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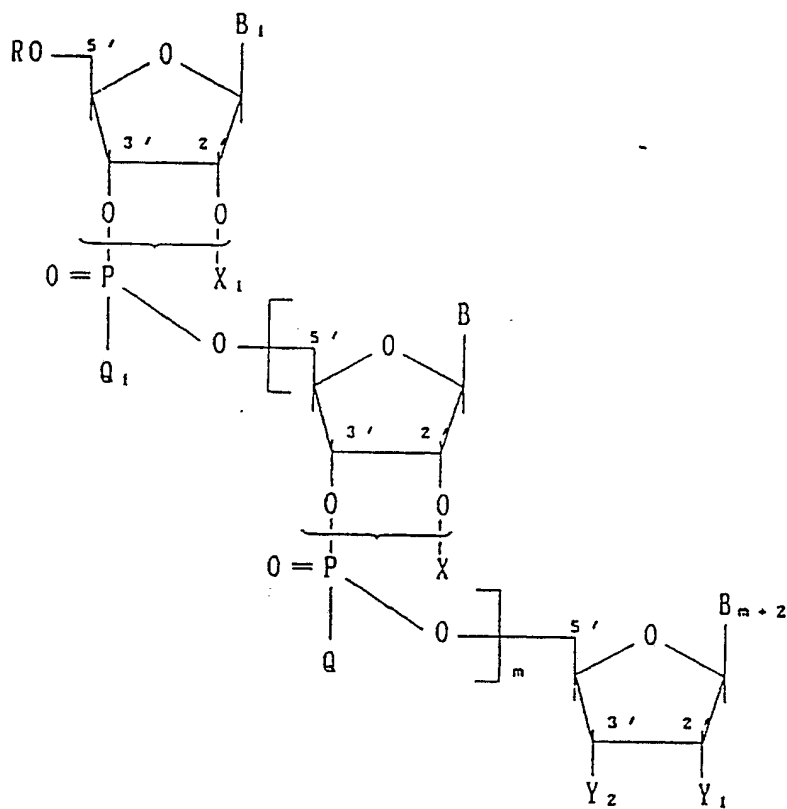
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⑤④ **Novel oligoribonucleotide derivatives and application thereof to antiviral agents.**

⑤⑦ The invention provides oligoribonucleotide derivatives represented by the general formula:

**EP 0 339 842 A2**



wherein m, B, Q, X, R, Y<sub>1</sub>, Y<sub>2</sub> and X are as defined in the specification. These derivatives inhibit the proliferation of the AIDS virus.

## NOVEL OLIGORIBONUCLEOTIDE DERIVATIVES AND APPLICATION THEREOF TO ANTIVIRAL AGENTS

The present invention relates to novel compounds, namely 2'-O-methylribooligonucleotide phosphorothioate derivatives, 2'-O-methylribooligonucleotide derivatives and oligoribonucleotide derivatives, which can inhibit proliferation of AIDS virus (HIV-1, HIV-2, etc.) causing AIDS (acquired immunodeficiency syndrome), to make AIDS virus avirulent, novel intermediates for producing the same and the application of these derivatives to drugs, for example, antiviral agents such as anti-AIDS drugs or the like.

AIDS (acquired immunodeficiency syndrome) was found in 1981 and it has been clarified that the cause lies in HIV (human immunodeficiency virus, hereafter simply referred to as AIDS virus) belonging to the family retro virus. The number of patients shows a yearly increase all over the world. According to the WHO's report, the number of patients exceeded 110,000 in the world as of August 31, 1988. In Japan, 90 patients are reported (as of August 31, 1988). Furthermore, if carriers infected with the virus but developing no AIDS yet are included, the number of patients will be more than 10,000,000 (estimated by WHO) in the world and 1048 in Japan (reported by the Ministry of Health and Welfare, as of August 31, 1988). A drug for completely curing AIDS that is a serious disease has not been found so far. 3'-Deoxy-3'-azidothymidine (hereafter simply referred to as "AZT") has been used in the clinical field as a drug for retarding the progress of AIDS and improving the clinical syndrome of the patient. However, side effect such as bone marrow impairment, etc. associated with its effectiveness are reported (cf. The New England Journal of Medicine, 317, 192-197, 1987). The mechanism of AZT is based on inhibition of the activity of reverse transcriptase possessed by the retro virus itself which is utilized, after invasion of the virus into host cells, to transcribe its genomic RNA onto DNA (Mitsuya, H., et al., Proc. Natl. Acad. Sci. USA, 82, 7096-7100, 1985) and it is thus probable that the mechanism would be related to the side effects.

On the other hand, it has been recently reported that phosphorothioate derivatives of deoxyoligonucleotides exhibit an anti-AIDS viral activity (cf. Proc. Natl. Acad. Sci. USA, 84, 7706-7710, 1987).

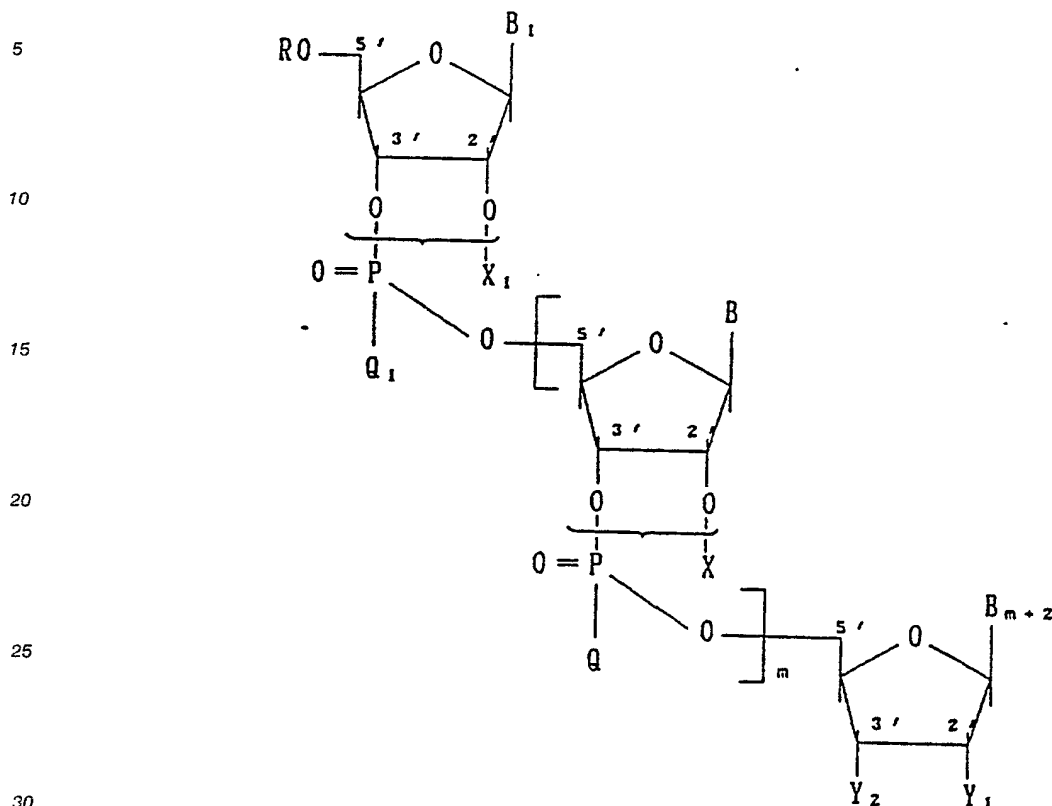
The AIDS virus has extremely frequent variations among its strains, in respect of its envelope protein. Therefore, it is extremely difficult to develop an effective vaccine and chemotherapeutic agents as described above are desired. However, nucleoside derivatives such as AZT conventionally used as chemotherapeutic agents exhibit anti-AIDS viral activity on one hand but on the other hand, involve serious side effects. Accordingly, it has been desired to develop drugs having minimized side effects and high practicability. For achieving the purpose, deoxyoligonucleotide phosphorothioate derivatives are at the stage of basic studies but it is desired to develop drugs having a more potent activity and provide such drugs at low costs.

As a result of extensive investigations to solve the foregoing problems, the present inventors have found that newly produced 2'-O-methyloligoribonucleotide derivatives and oligoribonucleotide derivatives could inhibit infection and proliferation of the AIDS virus to and in host cells and are applicable as drugs for treating AIDS, and have accomplished the present invention based on this finding.

Thus, the present invention is directed to:

- novel oligoribonucleotide derivatives represented by general formula (1) described below, and anti-AIDS viral compositions comprising the oligoribonucleotide derivatives as the effective ingredient;
- novel 2'-O-methyloligoribonucleotide phosphorothioate derivatives containing a sequence complementary to gene RNA of the AIDS virus (HIV-1, HIV-2, etc.) or DNA integrated into chromosome and represented by general formula (2) described below and anti-AIDS viral compositions comprising the derivatives as the effective ingredient;
- novel 2'-O-methyloligoribonucleotide derivatives represented by general formula (3) described below and anti-AIDS viral compositions comprising the derivatives as the effective ingredient;
- novel oligoribonucleotide derivatives represented by general formula (4) described below and anti-AIDS viral compositions comprising the derivatives as the effective ingredient; and,
- novel 2'-O-methyloligoribonucleotide derivatives represented by general formula (5) below and novel nucleoside derivatives represented by general formula (6) below.

## General formula (I)



wherein:

$m$  is an integer of 1 to 100 provided that when  $X_1$  and  $X$  are all substituted at the 2'-position and represent a hydrogen atom,  $m$  represents an integer of 4 to 50;

$B$  represents  $B_2, B_3, \dots, B_{(m+1)}$  and,  $B_1$  through  $B_{(m+2)}$ , which may be the same or different, each represents any one of hypoxanthine-9-yl, guanine-9-yl, adenine-9-yl, cytosine-1-yl, uracil-1-yl and thymine-1-yl (provided that when  $X_1$  and all of  $X$  are substituted at the 3'-position and represent a hydrogen atom, all of them do not represent adenine-9-yl);

$Q$  represents  $Q_2, Q_3, \dots, Q_{(m+1)}$  and,  $Q_1$  through  $Q_{(m+1)}$ , independently represents any one of a thio anion, an oxo anion, an alkyl group, an aryloxy group, an alkylamino group, an arylamino group, an alkylthio group and an arylthio group (provided that at least one of  $Q_1$  to  $Q_{(m+1)}$  represents a thio anion);

$X$  represents  $X_2, X_3, \dots, X_{(m+1)}$  and,  $X_1$  through  $X_{(m+1)}$ , which may be the same or different, each represents any one of a hydrogen atom, methyl group or an acyl group having 1 to 20 carbon atoms;

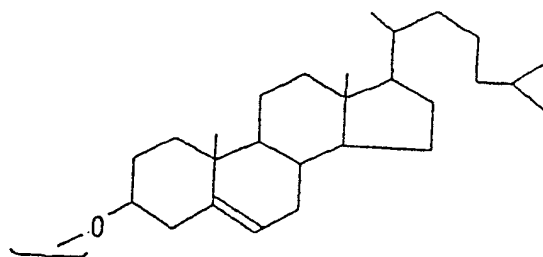
$R$  represents a hydrogen atom, an acyl group having 1 to 20 carbon atoms, a phosphoryl group which may optionally have a substituent, a thiophosphoryl group which may optionally have a substituent or an alkylaminophosphoryl group which may optionally have a substituent;

$Y_1$  and  $Y_2$  independently represents methoxy group, a carboxyl group having 1 to 20 carbon atoms, a hydrogen atom, hydroxyl group, an alkoxy group which may optionally have a substituent, a phosphoryloxy group which may optionally have a substituent, a thiophosphoryloxy group which may optionally have a substituent and an alkylaminophosphoryloxy group which may optionally have a substituent. Herein the alkyl represents a straight or branched chain hydrocarbon residue having 1 to 30 carbon atoms and the aryl represents a hydrocarbon residue having 6 to 30 carbon atoms including phenyl group.

In formula (1) described above,  $X$  can be bound to an oxygen atoms either at the 2'-position or at the 3'-position in the sugar moiety. In this case, another is bound to a phosphorus atom (P). A binding mode of the monomer at the sugar moiety may be any of 2'-5' bond and 3'-5' bond. Alternatively, the both binding modes may be possessed in the oligoribonucleotide.

Further in formula (1) described above, examples of the substituent on  $R, Y_1$  and  $Y_2$  include cyanoethyl

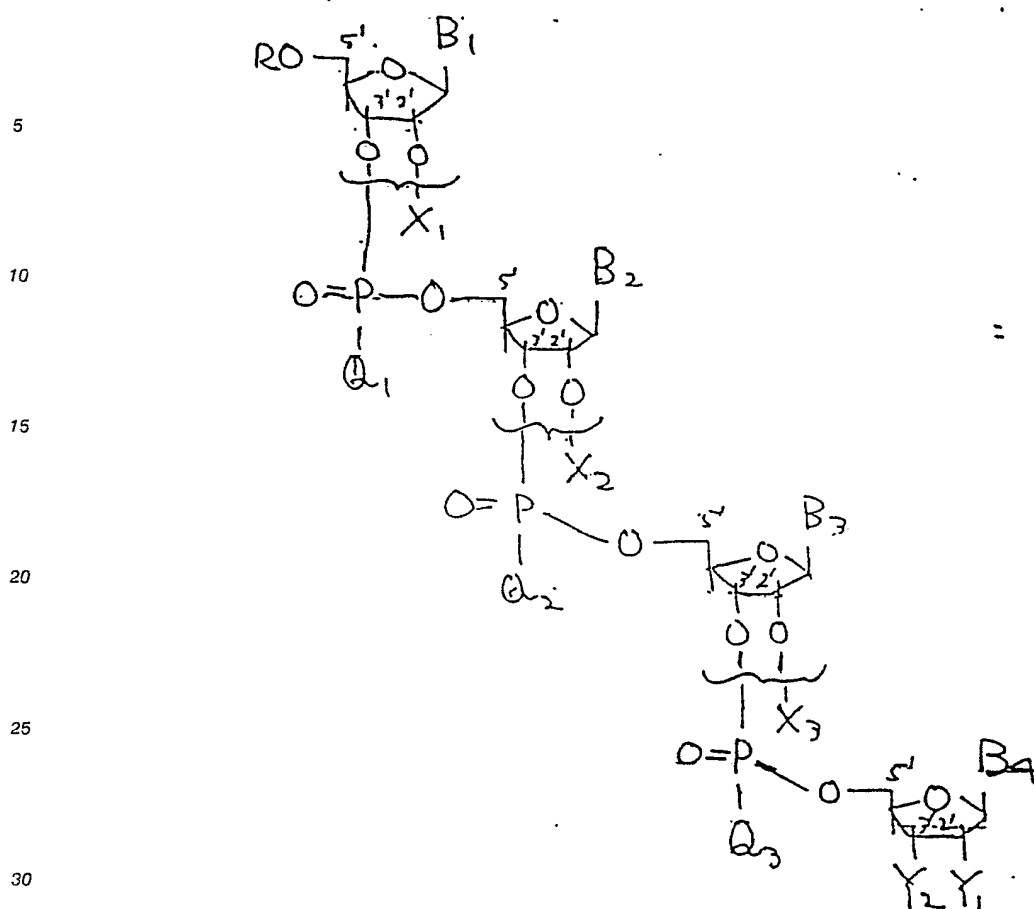
group, chlorophenyl group, monophosphoryl group, pyrophosphoryl group, a hydrocarbon residue having 1 to 20 carbon atoms, cholesteryl group and a group shown by  $J-(K-O-L)_E$ , which may be the same or different: provided that J represents:



- hydroxyl group or any one of the oligoribonucleotidyl group defined for the structural formula of said derivative according to claim 1, from which any one of RO, Y<sub>1</sub> and Y<sub>2</sub> is removed;  
 K represents any one of phosphoryl group, thiophosphoryl group and an alkylaminophosphoryl group having 1 to 10 carbon atoms;  
 L represents a hydrocarbon residue having 1 to 20 carbon atoms; and,  
 E represents an integer of 1 to 10.

The nucleotide sequence may not necessarily be complementary to the sequence of genomic RNA of AIDS virus or DNA integrated into chromosome.

- In the formula described above, B<sub>1</sub> through B<sub>(m+2)</sub>, Q<sub>1</sub> through Q<sub>(m+1)</sub> and X<sub>1</sub> through X<sub>(m+1)</sub> are shown from the 5'-end in the order of 1, 2, 3, 4, ... (m+1). For example, when m is 2, B<sub>1</sub> to B<sub>(m+2)</sub> are, in the order from the 5'-end, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>4</sub>; Q<sub>1</sub> to Q<sub>(m+1)</sub> are, in the order from the 5'-end, Q<sub>1</sub>, Q<sub>2</sub>, Q<sub>3</sub>; and X<sub>1</sub> through X<sub>(m+1)</sub> are, in the order from the 5'-end, X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>; and the following structural formula is presented:



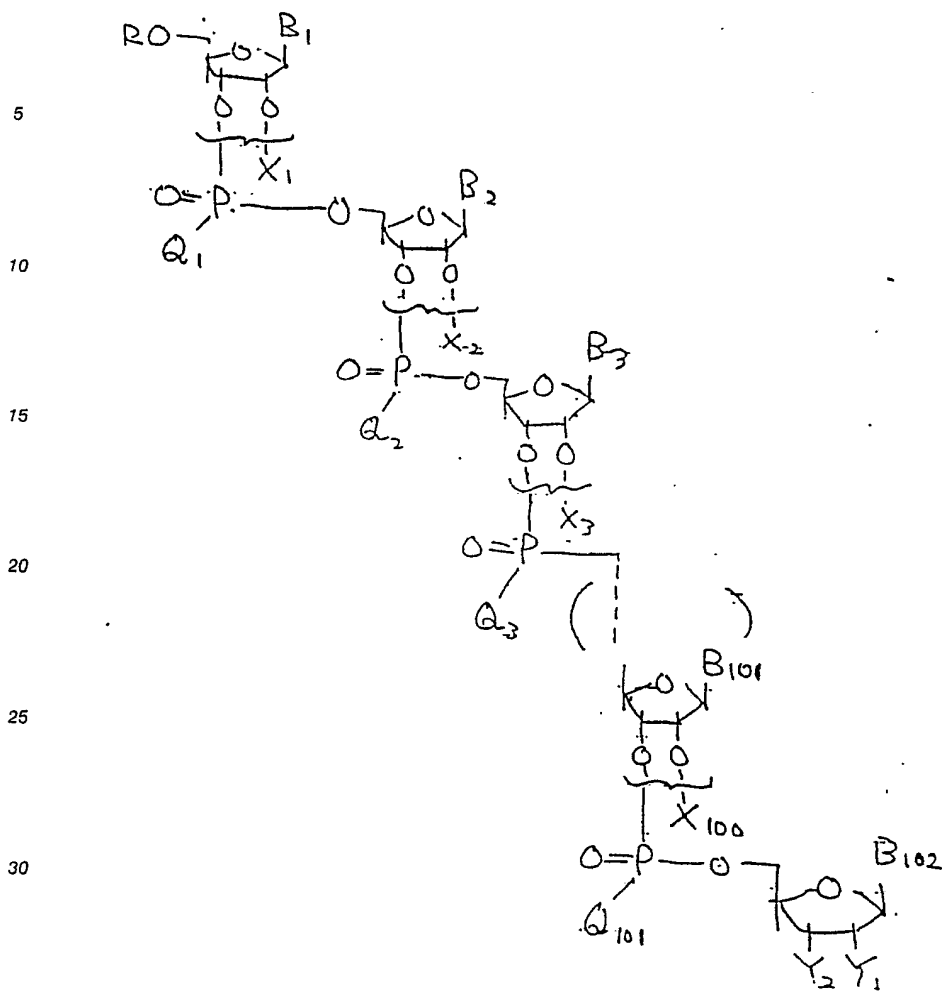
Likewise, when  $m$  is 100,  $B_1$  to  $B_{(m+2)}$  are, in the order from the 5'-end,  $B_1, B_2, B_3, \dots B_{100}, B_{101}, B_{102}$ ;  
 35  $Q_1$  to  $Q_{(m+1)}$  are, in the order from the 5'-end,  $Q_1, Q_2, Q_3, \dots Q_{100}, Q_{101}$ ; and  $X_1$  through  $X_{(m+1)}$  are, in the  
 order from the 5'-end,  $X_1, X_2, X_3, \dots X_{100}, X_{101}$ ; and the following structural formula is presented:

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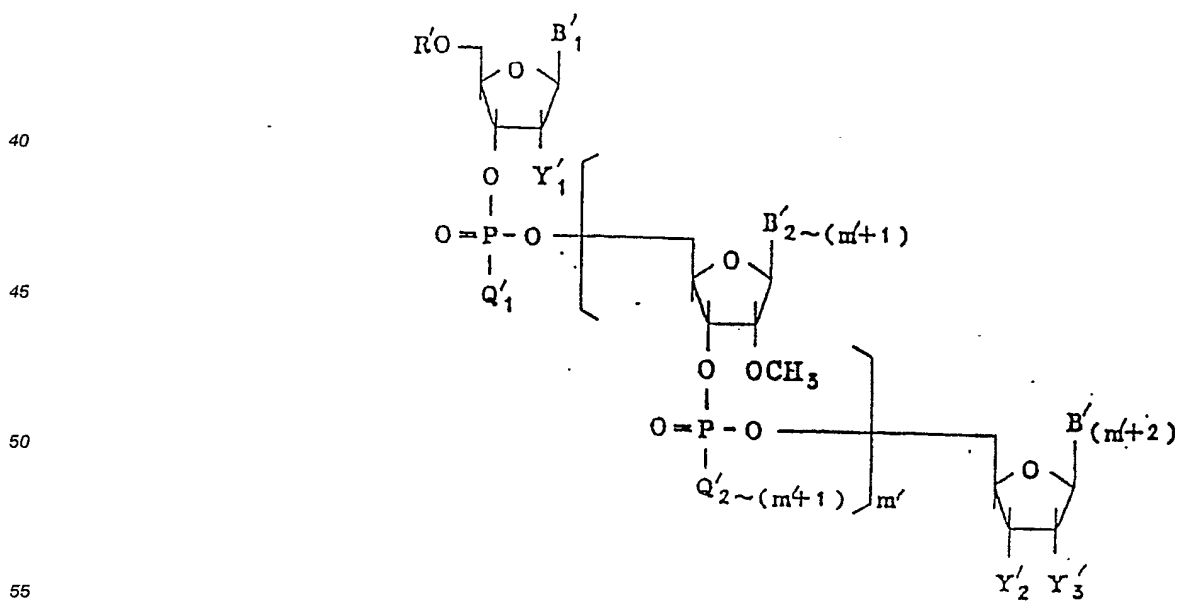
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General formula (2)



wherein:

$m'$  is an integer of 1 to 50;

$B'_1$  through  $B'_{(m'+2)}$ , which may be the same or different, each represents any one of adenine-9-yl (A), guanine-9-yl (G), cytosine-1-yl (C), uracil-1-yl (U), thymine-1-yl (T) and hypoxanthine-9-yl (H);

5  $Q'_1$  represents any one of a thio anion ( $S^-$ ), an oxo anion ( $O^-$ ), an alkyl group, an alkyloxy group, an aryloxy group, an alkylamino group, an arylamino group, an alkylthio group and an arylthio group; at least one of  $Q'_2$  through  $Q'_{(m'+1)}$  represents a thio anion ( $S^-$ ) and the balance is either a thio anion or an oxo anion ( $O^-$ );

10  $Y'_1$  through  $Y'_3$  independently represents methoxy group, an alkyloxy group which may optionally have a substituent, hydroxyl group, an alkylsilyl group and a hydrogen atom;

$R'$  represents a hydrogen atom, a phosphoryl group or thiophosphoryl group which may optionally have a substituent. Herein the alkyl represents a straight or branched chain hydrocarbon residue having 1 to 30 carbon atoms and the aryl represents a hydrocarbon residue having 6 to 30 carbon atoms including phenyl group. Examples of the substituent include cyanoethyl group, chlorophenyl group and a hydrocarbon residue having 1 to 10 carbon atoms.

15 In the formula described above,  $B'$  and  $Q'$  are shown in the order of 1, 2, 3, 4, ... ( $m' + 2$ ). For example, when  $m'$  is 2,  $B'_2$  to  $B'_{(m'+1)}$  are, in the order from the 5'-end,  $B'_2$ ,  $B'_3$ ;  $Q'_2$  to  $Q'_{(m'+1)}$  are in the order from the 5'-end,  $Q'_2$ ,  $Q'_3$ ; and the following structural formula is presented:

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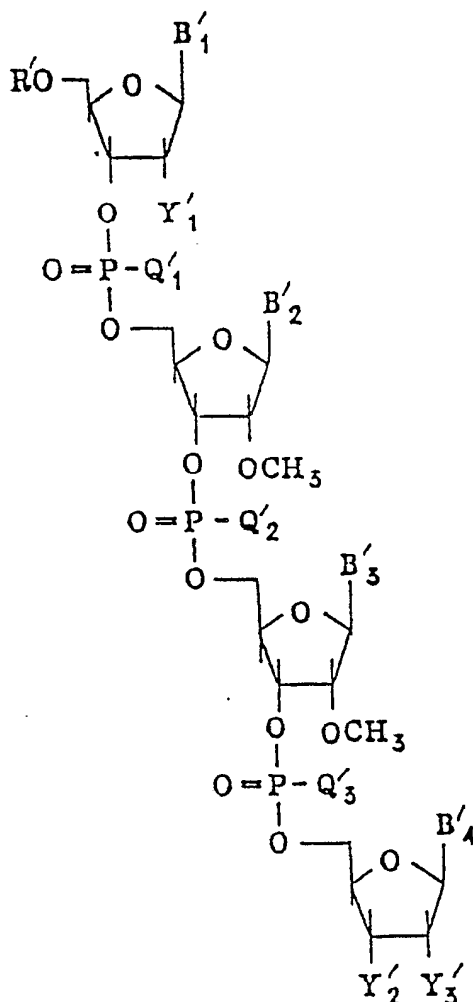
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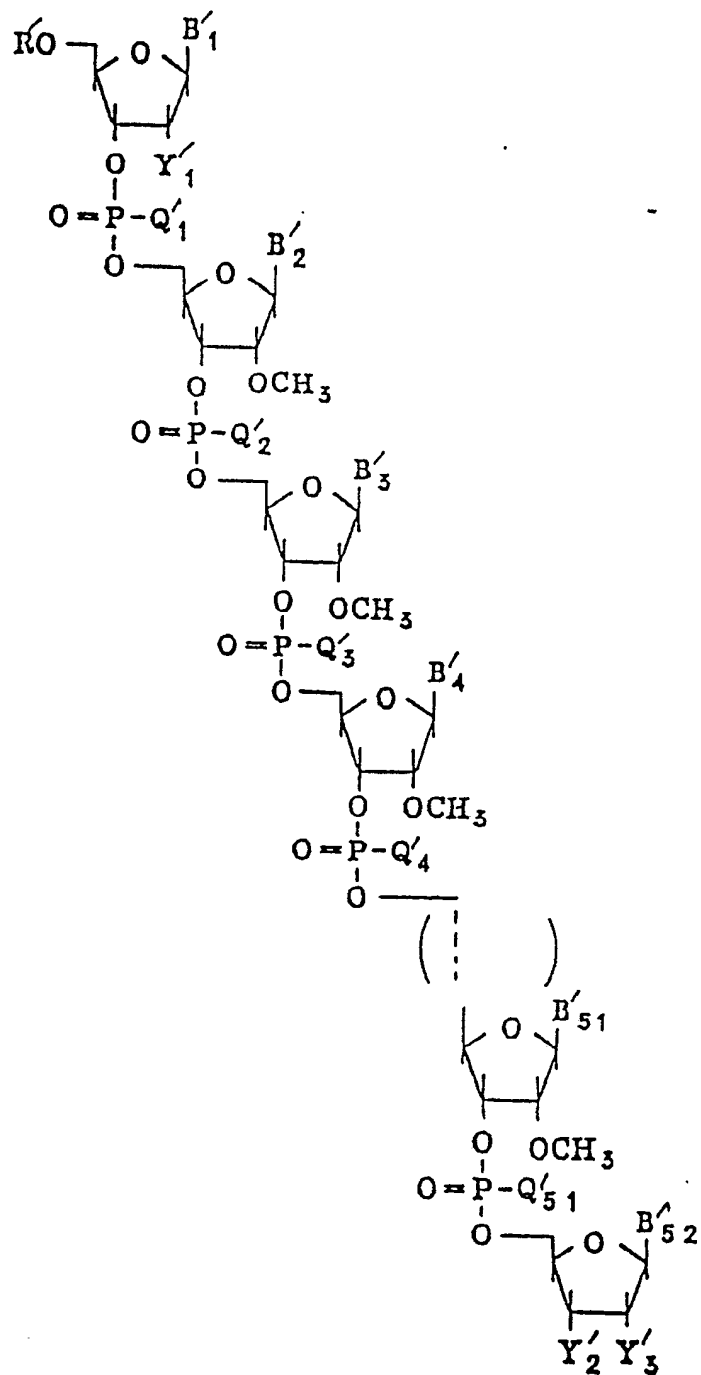
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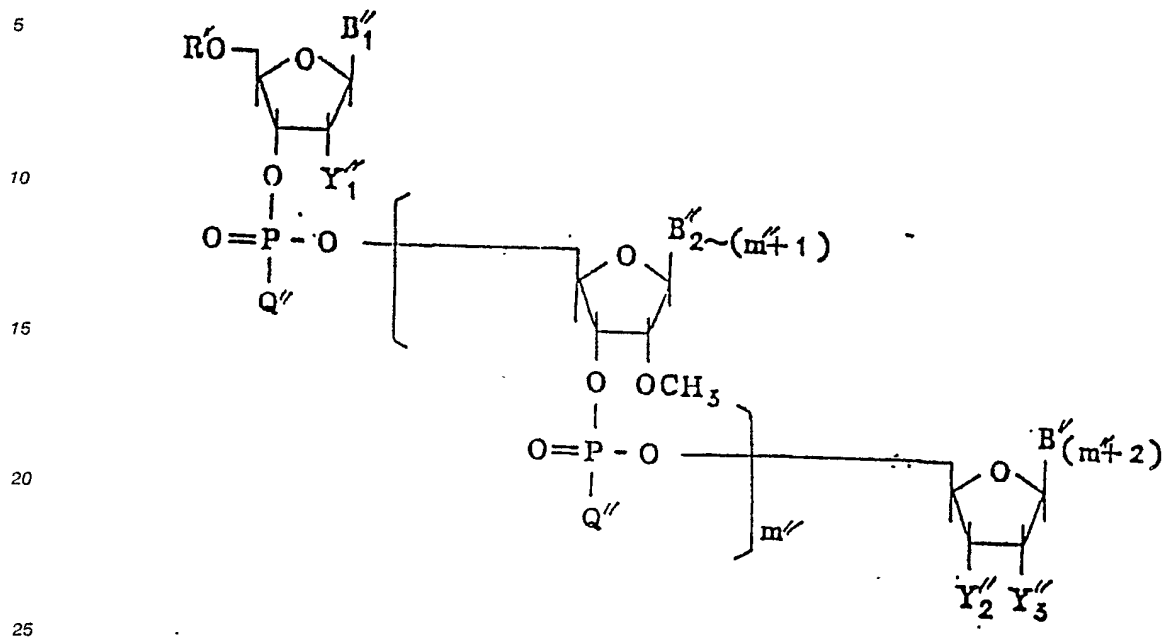
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Likewise, when  $m'$  is 50,  $B'_2$  to  $B'_{(m'+1)}$  are, in the order from the 5'-end,  $B'_2$ ,  $B'_3$ ,  $B'_4$ , ...  $B'_{50}$ ,  $B'_{51}$ ;  $Q'_2$  to  $Q'_{(m'+1)}$  are, in the order from the 5'-end,  $Q'_2$ ,  $Q'_3$ ,  $Q'_4$ , ...  $Q'_{50}$ ,  $Q'_{51}$ ; and the following structural formula is presented:





## General formula (3)



wherein:

$m''$  is an integer of 1 to 50;

30  $B''_1$  through  $B''_{(m''+2)}$ , which may be the same or different, each represents any one of adenine-9-yl (A), guanine-9-yl (G), cytosine-1-yl (C), uracil-1-yl (U), thymine-1-yl (T) and hypoxanthine-9-yl (H);

$Q''$  contains at least one thio anion and represents any one of a thio anion ( $S^\ominus$ ), an oxo anion ( $O^\ominus$ ), an alkyl group, an alkoxy group, an aryloxy group, an alkylamino group, an arylamino group, an alkylthio group and an arylthio group;

35  $Y''_1$  through  $Y''_3$  independently represents methoxy group, an alkoxy group which may optionally have a substituent, hydroxyl group, an alkylsilyl group and a hydrogen atom;

$R''$  represents a hydrogen atom or a phosphoryl group which may optionally have a substituent.

Herein the alkyl represents a straight or branched chain hydrocarbon residue having 1 to 30 carbon atoms and the aryl represents a hydrocarbon residue having 6 to 30 carbon atoms including phenyl group.

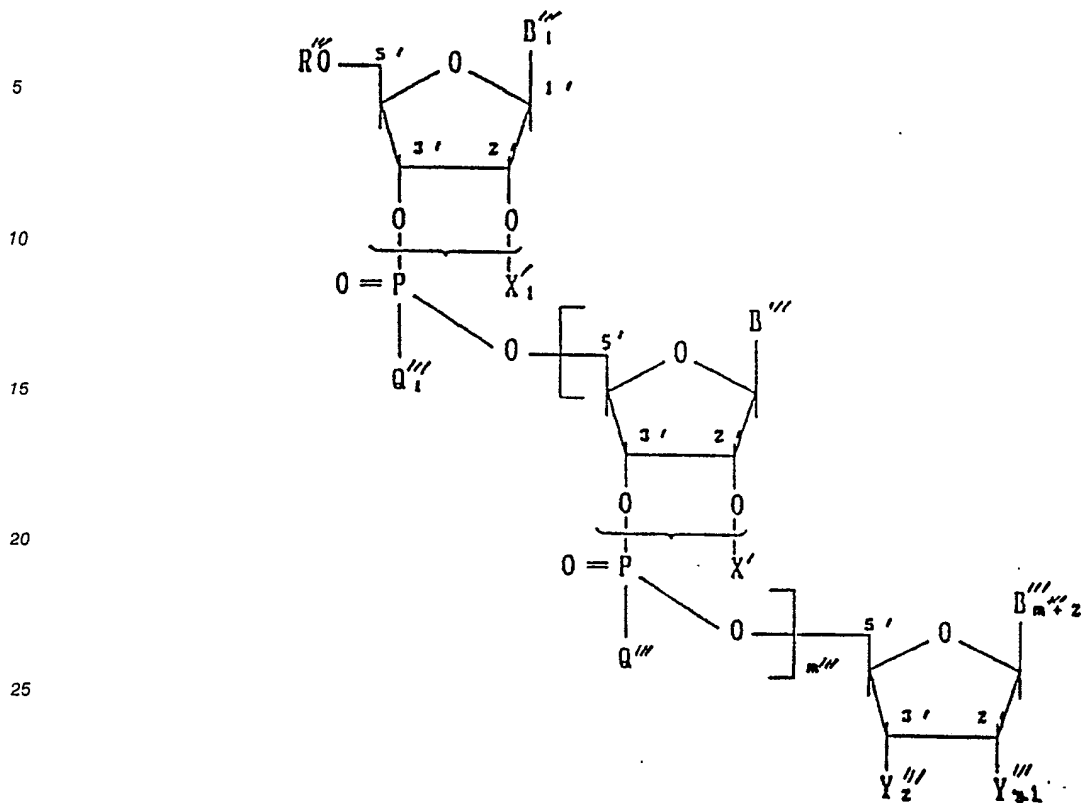
40 Examples of the substituent include cyanoethyl group, chlorophenyl group and a hydrocarbon residue having 1 to 10 carbon atoms. The nucleotide sequence may not necessarily be complementary to the sequence of genomic RNA of AIDS virus or DNA integrated into chromosome.

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## General formula (4)



wherein:

$m''$  is an integer of 1 to 100 (provided that when  $X'_1$  and  $X'_2$  are all substituted at the 2'-position and represent a hydrogen atom,  $m''$  represents an integer of 4 to 50);

$B''$  represents  $B''_2, B''_3, \dots, B''_{(m''+1)}$ , provided that all of them do not represent adenine-9-yl; and,  $B''_1$  through  $B''_{(m''+2)}$ , which may be the same or different, each represents any one of hypoxanthine-9-yl, guanine-9-yl, adenine-9-yl, cytosine-1-yl, uracil-1-yl and thymine-1-yl;

$Q''$  represents  $Q''_2, Q''_3, \dots, Q''_{(m''+1)}$  and, at least one of  $Q''_1$  to  $Q''_{(m''+1)}$  represents a thio anion.  $Q''_1$  through  $Q''_{(m''+1)}$ , independently represents any one of a thio anion, an oxo anion, an aryloxy group, an alkylamino group, an arylamino group, an alkylthio group and an arylthio group;

$X'$  represents  $X'_2, X'_3, \dots, X'_{(m''+1)}$  and,  $X'_1$  through  $X'_{(m''+1)}$ , which may be the same or different, each represents a hydrogen atom or methyl group (provided that when all of  $X'_1$  through  $X'_{(m''+1)}$  are substituted at the 2'-position, all of them do not represent methyl group);

$R''$  represents any one of a hydrogen atom, an acyl group having 1 to 20 carbon atoms and a phosphoryl group which may optionally have a substituent;

$Y''_1$  and  $Y''_2$  independently represents any one of methoxy group, a carboxyl group having 1 to 20 carbon atoms, an alkyloxy group which may optionally have a substituent, hydroxyl group, an alkylsilyl group, a hydrogen atom and a phosphoryl group which may optionally have a substituent.

Herein the alkyl represents a straight or branched chain hydrocarbon residue having 1 to 30 carbon atoms and the aryl represents a hydrocarbon residue having 6 to 30 carbon atoms including phenyl group. Examples of the substituent include cyanoethyl group, chlorophenyl group and a hydrocarbon residue having 1 to 20 carbon atoms.

In the formula described above, the oxygen atoms at the 2'-position and at the 3'-position in the sugar moiety of nucleoside monomer indicate that when one is  $X'_1$ , another is bound to phosphorus atom (P). A binding mode of the monomer at the sugar moiety may be any of 2'-5' bond and 3'-5' bond. Alternatively, the both binding modes may be possessed in the oligoribonucleotide.

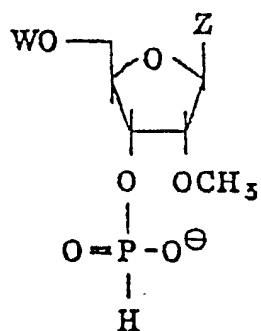
The nucleotide sequence may not necessarily be complementary to the sequence of genomic RNA of AIDS virus or DNA integrated into chromosome.

## General formula (5)

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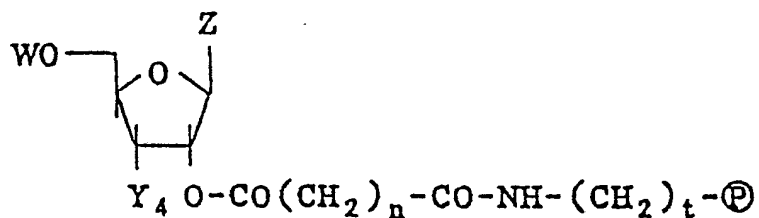
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## General formula (6)

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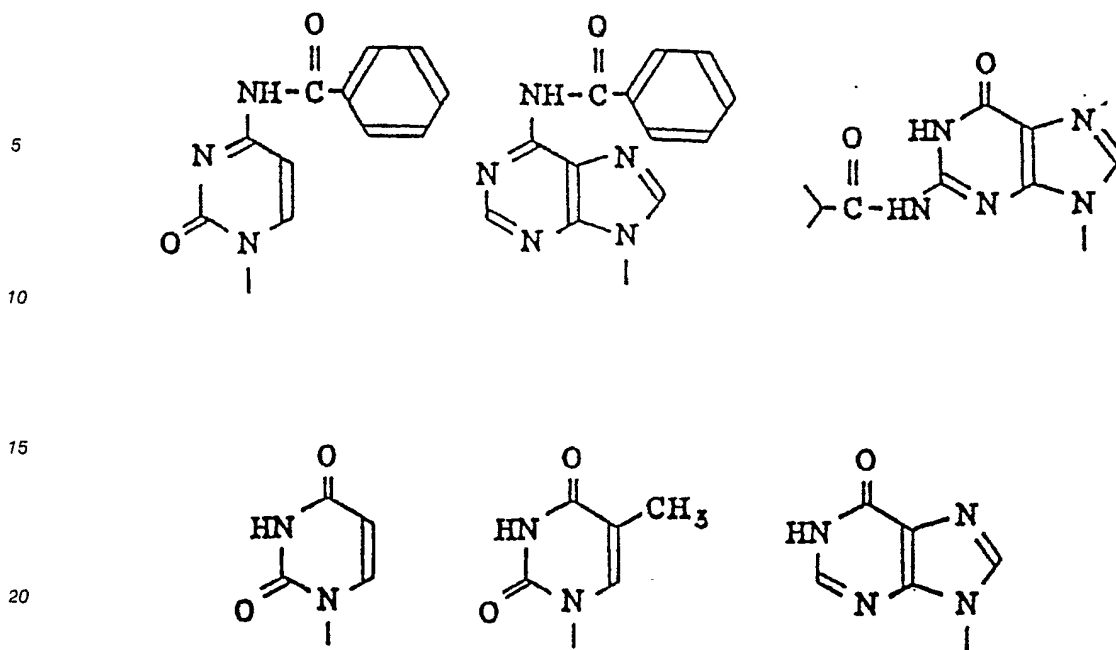
In the general formulae (5) and 6), W represents monomethoxytrityl group or dimethoxytrityl group; Y<sub>4</sub> represents methoxy group, an alkyloxy group which may optionally have a substituent or a hydrogen atom; n and t, which may be different from each other, or independently, each represents an integer of 1 to 30;  $\oplus$  represents any one of a controlled pore glass derivative, a polystyrene derivative or a silica gel derivative; and Z represents a substituent represented by any one of general formulae below:

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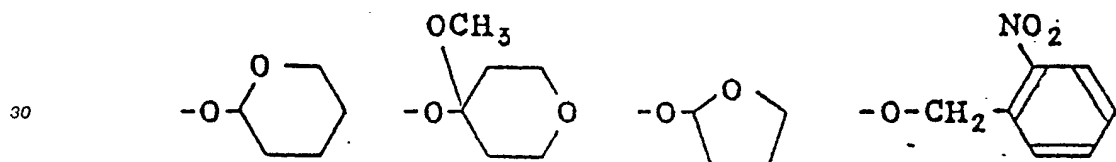
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25 The carbon atom number of the alkyl moiety in the alkyloxy group is 1 to 10. Examples of the alkyloxy group having a substituent include:



etc.

35 With respect to the antiviral activity of oligodeoxynucleotide, an example of using oligodeoxynucleotide for inhibiting proliferation of Raus sarcoma virus without converting it into any derivative is reported (Zamecnik, P.C., Proc. Natl. Acad. Sci. USA, 75, 280-284, 1978). On the other hand, it is reported that oligodeoxynucleotide is rapidly decomposed in culture of mammal cells, cell extracts or sera (Wickstrom, E., Journal of Biochemical and Biophysical Methods, 13, 97-102, 1986). Accordingly, in order to expect a

40 more effective antiviral activity of oligodeoxynucleotide, it is desired that the oligodeoxynucleotide be converted into such derivatives that are not decomposed. It is reported that for example, derivatives obtained by converting the phosphodiester in oligodeoxynucleotide into methylphosphonate inhibit proliferation of herpes simplex virus type I (Smith, C.C., et al., Proc. Natl. Acad. Sci. USA, 83, 2787-2791, 1986). It is also reported that derivatives obtained by converting the phosphodiester in oligodeoxynucleotide into phosphorothioate have an improved stability (Stein, C.A., Nucleic Acids Res., 16, 3209-3221, 1988). It is further reported that these derivatives inhibit proliferation of AIDS virus (Matsukura, M., et al., Proc. Natl. Acad. Sci. USA, 84, 7706-7710, 1987). It is further reported that by leading to phosphorothioate derivatives in double stranded poly RNA or an interferon inducer, antiviral activity against bovine vesicular stomatitis virus is enhanced (De Clercq, E., et al., Virology, 42, 421-428, 1970).

50 On the other hand, it is known that 2'-O-methylribooligonucleotide takes a structure of the 2'-hydroxy group in the sugar moiety of RNA being methylated and has the property showing resistance to various enzymes (nucleases) for decomposing DNA or RNA (Gray, M.W., et al., Biochem. Biophys. Acta, 134, 243, 1967; Dunlap, B.E., et al., Biochemistry, 10, 2581-2587, 1971). Furthermore, 2'-O-methylribooligonucleotide has the property that it forms a stable duplex with RNA having its complementary sequence and the

55 stability is significantly better than the stability of DNA-RNA complementary double strand having the same nucleotide sequence (Inoue, H., et al., Nucleic Acids Res., 15, 6131-6148, 1987). It is also known that 2'-O-methylribooligonucleotide is used for site-specific cleavage of RNA, utilizing the property resistant to decomposition from RNase H

(Inoue, H., et al., FEBS Letters, 215, 327-330, 1987). In addition, 2'-O-methylribooligonucleotide is used to produce a unidirectional deletion mutant of DNA, utilizing the property showing significant resistance to DNase Ba131 nuclease as compared to DNA (Mukai, S., et al., Nucleic Acids Symposium Series, No. 19, 117-120, 1988). The foregoing findings are based on the characteristics that 2'-O-methylribooligonucleotide forms a stable duplex with RNA having its complementary nucleotide sequence and shows resistance various enzymes (nucleases) for decomposing DNA or RNA. Moreover, it was reported in the past that poly-2'-O-methylribooligonucleotide inhibited the progress of cancer in mice caused by retro virus, while its mechanism was clearly unknown (Tennant, R.W., et al., Proc. Natl. Acad. Sci., USA, 71, 3167-3171, 1974). In addition, 2'-O-methylribooligonucleotide can be prepared as in oligoribonucleotide using less expensive raw materials than in deoxyoligonucleotide.

With respect to the structure and properties of AIDS virus, the following findings have been made according to recent studies. That is, AIDS virus is retro virus having a single stranded RNA of about 9000 nucleotides in length as the substance of gene and has reverse transcriptase. Its nucleotide sequence (primary structure) of gene is already reported (Ratner, L., et al., Nature, 313, 277-284, 1985; Muesing, M.A., et al., Nature, 313, 450-458, 1985; Sanchez-Pescador, R., et al., Science, 227, 484-492, 1985; Wain-Hobson, S., et al., Cell, 40, 9-17, 1985). Target cells of infection are helper/inducer cells which are T4 (antigen detected with a monoclonal antibody) positive as surface antigen among human lymphocytes and after infection, cause these cytopathic effects and break down. The virus after adsorption to and invasion into the cells changes to linear double stranded viral DNA by reverse transcriptase possessed by themselves utilizing tRNA<sup>Lys</sup> as a primer, transfers into the nucleus to take a circular structure and is integrated into host cell chromosomal DNA to become provirus. Transcription from the provirus is effected by cell-derived RNA polymerase II as in messenger RNA (hereafter simply referred to as "mRNA") of host cells, whereby the provirus is translated into viral protein. This procedure is regulated by at least two gene products, i.e., tat (transactivator) and rev (regulator of expression of virion proteins) encoded by the virus per se. tat works as a transcription activation factor for virus upon the region called TAR element (transacting responsive element) immediately after the initiation site of transcription (Rosen, C.A., et al., Cell, 41, 813-823, 1985). The transcribed viral RNA becomes in part genome RNA encapsulated into virion and is in part utilized as mRNA for expressing gene. Several species of mRNA are present; RNA in full length for expressing viral antigen and reverse transcriptase, mRNA for expressing envelope glycoprotein which is shortened by being spliced once, mRNA which is further shortened by being spliced at least twice in order to express genes such as tat, rev, etc., and the like.

As described above, the present invention has been successful for inhibiting infection with and proliferation of AIDS virus by further derivating the phosphate moiety, taking advantages of these 2'-O-methylribooligonucleotide and oligoribonucleotide. It has also been made clear that the inhibitory effect can be exhibited in any binding mode of 2'-5' bond and 3'-5' bond in the monomer of these oligomers, that is, 3'-O-methylnucleoside may be its constituent component. Accordingly, the 2'-5' bond and the 3'-5' bond in the sugar may be both contained in the monomer of the oligomer molecule. Further, the 2'- (or 3'-) hydroxy group may be all or in part methylated or acylated. The sugar at the 3'- and 5'-termini in the oligonucleotide may optionally have a substituent as shown in general formulae (1) through (4).

As described above, the compound of the present invention is in the form that the phosphate in the oligomer is derivated. As a result of various investigations, it has been found that the derivatives containing a thiophosphate bond is particularly effective and the thiophosphate bond is not necessary for all of the phosphodiester bonds in the oligomer molecule. It has also been found that the inhibitory effect can be exhibited in any of the case that these nucleotide sequences are complementary to those of genomic RNA for AIDS virus or DNA integrated into chromosome and in the case that the nucleotide sequences are not necessary complementary.

In the present invention, the oligomer having a complementary sequence should preferably take as the target a region that plays an important role on viral gene. For example, the derivative (Compound VI) recited in claim 15, the derivative (Compound VII) recited in claim 16, the derivative (Compound VIII) recited in claim 17, the derivative (Compound IX) recited in claim 18 and the derivative (Compound XII) recited in claim 21 are all sequences complementary to the region having a nucleotide sequence:

5' U U U A U C C A U U U C A G A A U U G G G U C U 3'

which is present after 5300th (the initiation site of transcription is counted as the first) of viral genomic RNA or mRNA, and the region in which cleavage and ligation of the first splicing of mRNA occur. Therefore, by obstructing the same, it can be expected that expression of envelope protein or genes such as tat, rev, etc. is inhibited. Furthermore, the derivative (Compound X) recited in claim 19 is complementary to a nucleotide sequence:

5' C A G U G G C G C C C G A A C A G G G A C 3'

including the primer-tRNA binding site present in the region for initiating reverse transcription of viral genomic RNA. Accordingly, hindrance of initiating transcription that is the first stage of replicating viral gene can be expected.

Further, the derivative (Compound XI) recited in claim 20 is complementary to a nucleotide sequence in  
 5 TAR element upon which the tat product:  
 5' C A G A U C U G A G C C U G G G A G C U C 3'  
 present around the 5'-terminal of viral genomic RNA or mRNA works;

The derivative (Compound XXVII) recited in claim 3 is complementary to a nucleotide sequence present downstream from the primer binding site on viral genomic RNA and, it can be expected to inhibit  
 10 transcription and replication of virus.

The derivative (Compound XXVIII) recited in claim 4 is complementary to a nucleotide sequence right upstream gag gene initiation codon.

The derivative (Compound XXIX) recited in claim 5 is complementary to a nucleotide sequence in gag gene and, it can be expected to inhibit translation of gag protein.

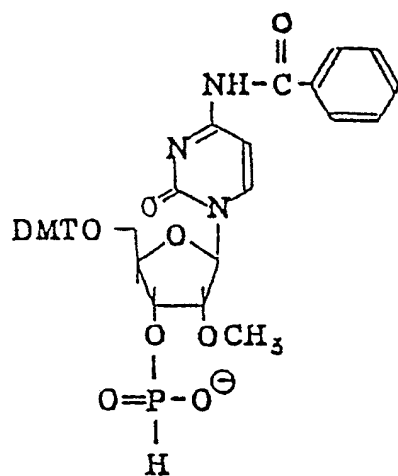
15 It is noted that all these derivatives show a potent anti-AIDS viral activity, although mechanism on the activity has not been experimentally proven in detail.

On the other hand, as is described in publications (cf., Matsukura, M., et al., Proc. Natl. Acad. Sci. USA, 84, 7706-7710, 1987; Agrawal, S., et al., Proc. Natl. Acad. Sci. USA, 85, 7709-7083, 1988) regarding anti-  
 AIDS viral activity using oligodeoxynucleotide derivatives, it is reported that oligodeoxynucleotide derivatives  
 20 not complementary to the nucleotide sequence of AIDS viral genomic RNA or DNA integrated into chromosome also possess anti-AIDS viral activity, like the complementary derivatives.

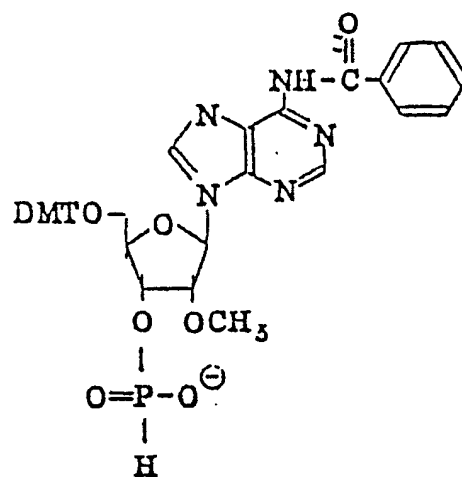
On the other hand, the compounds represented by general formulae (1), (3) and (4) in the present invention which can be produced using less expensive raw materials than in oligodeoxynucleotide derivatives exhibit the anti-AIDS viral activity, although these compounds are not necessarily complementary to  
 25 the nucleotide sequence of AIDS viral genomic RNA or DNA integrated into chromosome. It has been made clear that among the compounds represented by general formula (1), for example, the derivatives (Compound XXX) recited in claim 6, the derivatives (Compound XXXI) recited in claim 7, the derivatives (Compound XXXII) recited in claim 8, the derivatives (Compound XXXIII) recited in claim 9, the derivatives (Compound XXXIV) recited in claim 10, the derivatives (Compound XXXV) recited in claim 11 and the  
 30 derivatives (Compound XXXVI) recited in claim 12 exhibit the anti-AIDS viral activity, although these compounds are not complementary to the nucleotide sequence of AIDS viral genomic or DNA integrated into chromosome. It has also been made clear that for example, the derivatives (Compound XV) recited in claim 25, the derivatives (Compound XVI) recited in claim 26, the derivatives (Compound XVII) recited in claim 27 and the derivatives (Compound XVIII) recited in claim 28, among the compounds represented by  
 35 general formula (3), and among the compounds represented by general formula (4), for example, the derivatives (Compound XXIII) recited in claim 31, the derivatives (Compound XXIV) recited in claim 32, the derivatives (Compound XXV) recited in claim 33 and the derivatives (Compound XXVI) recited in claim 34 exhibit the anti-AIDS viral activity, although these compounds are not complementary to the nucleotide sequence of AIDS viral genomic RNA or DNA integrated into chromosome.

40 On the other hand, the compounds in the present invention have low molecular weights unlike high molecular weight double stranded RNA known as a potent interferon inducer (cf., Lanpson, G.P., et al., Proc. Natl. Acad. Sci. USA., 58, 782, 1967; Field, A.K., et al., Proc. Natl. Acad. Sci. USA., 58, 1004, 1967) and derivatives thereof (De Clercq, E., et al., Virology, 42, 421-428, 1970). The compounds in the present invention are also clearly distinguished over 2'-5' oligoA all composed of adenosines and containing 2'-5'  
 45 bond which is a protein synthesis inhibitor found in cells treated with interferon (cf., Kerr, I.M., Proc. Natl. Acad. Sci. USA., 75, 256, 1978) and derivatives thereof.

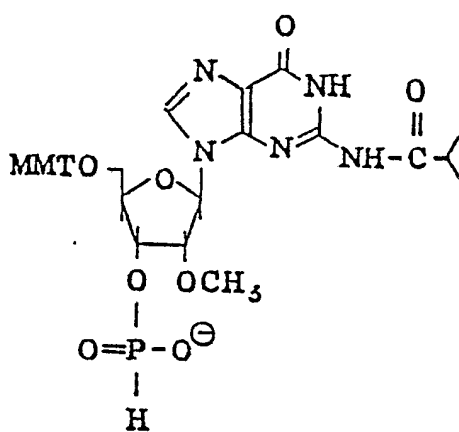
The oligoribonucleotide derivatives of the present invention can be produced by known methods such as the amidite method (cf., Matteucci, M.D., et al. Tetrahedron Lett., 22, 719, 1980), the H-phosphonate method (cf., Froehler, B.C. et al. Tetrahedron Lett., 27, 469, 1986; Garegg, P.J., et al., Tetrahedron Lett., 27,  
 50 4055, 1986) and the like. The 2'-O-methylribonucleotide derivatives represented by general formula (5) which can be provided as raw materials for producing the 2'-O-methylribonucleotide derivatives according to the H-phosphonate method can be produced by leading to the protected 2'-O-methylribonucleoside derivatives by known methods (cf., Robins, M.J., et al., J. Org. Chem., 39, 1891, 1974; Heikkila, J., et al., Acta Chem. Scand., B36, 715, 1982; Inoue, H., et al., Nucleic Acids Research, 15, 6131,  
 55 1987) and then reacting with phosphorus trichloride by known method (cf., Froehler, B.C., et al., Tetrahedron Letters, 27, 469-472, 1986; Garegg, P.J., et al., Tetrahedron Letters, 27, 4051-4054, 1986). According to the process, Compounds I, II, III, IV and V shown below were produced.



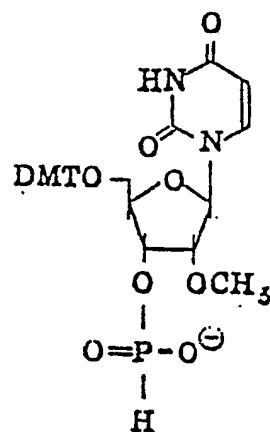
(Compound I)



(Compound II)

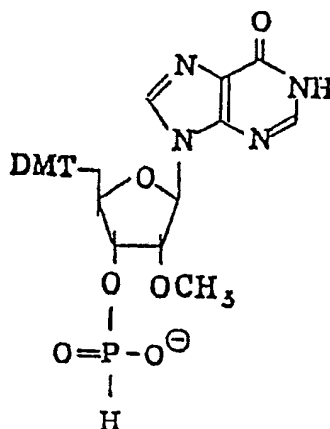


(Compound III)



(Compound IV)





(Compound V)

wherein DMT and MMT represent dimethoxytrityl group and monomethoxytrityl group, respectively.

Further the 2'-O-methylribonucleotide derivatives represented by general formula (5) can also be produced by reacting the protected 2'-O-methylribonucleoside derivatives with phosphorous acid in the presence of a condensation reagent such as triisopropylbenzenesulfonyl chloride or pivaloyl chloride by known methods (cf., Hata, T., et al., J. Am. Chem. Soc., 96, 7363, 1974; Sekine, M., et al., Tetrahedron Letters, 29, 1037-1040, 1988). Furthermore, the protected 3'-O-methylnucleoside derivatives are produced according to known methods (cf., Robins, M.J., et al., J. Org. Chem., 39, 1891, 1974; Heikkila, J., et al., Acta Chem. Scand., B36, 715, 1982) and the derivatives can be led to the 3'-O-methylnucleoside-H-phosphonate derivatives by the process described above, which can be provided as raw materials for producing the oligoribonucleotide derivatives represented by general formulae (1) and (4).

In the oligoribonucleotide derivatives represented by general formulae (1) and (4) wherein all or any of the substituents shown by  $X_1 - X_{m+1}$  or  $X'_1 - X'_{m''+1}$  are hydrogen, raw materials for producing the derivatives are, for example, protected 2'(or 3')-O-(tert-butyl dimethylsilyl)ribonucleosides, which can easily be produced by a known method (Ogilvie, K.K., Can. J. Chem., 51, 3799, 1973); these derivatives can be led to the H-phosphonate derivatives in a manner similar to described above.

The novel nucleoside derivatives represented by general formula (6) can be produced, for example, by a known method (cf., Miyoshi, K., et al., Nucleic Acids Res., 8, 5491, 1980) using the protected 3'-O-methyl (3'-O-alkyl or 3'-deoxy)nucleosides.

Using the protected novel 2'-O-methylribonucleotide derivatives, protected 3'-O-methylribonucleotide derivatives, protected 2'(or 3')-O-(tert-butyl dimethylsilyl)ribonucleotide derivatives and novel nucleoside derivatives produced by the foregoing processes, the 2'-O-methylribooligonucleotide derivatives and oligoribonucleotide derivatives can be produced, for example, by known methods (cf., Froehler, B.C., et al., Tetrahedron Letters, 27, 469-472, 1986; Garegg, P.J., et al., Tetrahedron Letters, 27, 4051-4054, 1986). That is, the nucleotide derivative elements are sequentially condensed novel nucleoside derivatives as the polymer support, having acyl chloride to elongate the chain. Then, oxidation is performed using various oxidizing agents such as sulfur ( $S_8$ ), iodine ( $I_2$ ) or an alkylamine/carbon tetrachloride, etc. followed by removal of the protective group and purification.

Further in the compounds represented by general formulae (1), (2) and (4) wherein the substituents shown by Q, Q' and Q'' are thio anions or thio anions and oxo anions and in the compounds represented by general formula (3), these compounds can also be produced by the amidite method (cf., Matteucci, M.D., et al., Tetrahedron Lett., 22, 719, 1980; Shibahara, S., et al., Nucleic Acids Res., 15, 4403, 1987; Usman, N., et al., J. Am. Chem. Soc., 109, 7845, 1987). In this case, the monomer may be oxidized with ( $S_8$ ) or iodine ( $I_2$ ) in the oxidation step after the condensation and then subjected to chain elongation followed by removal of the protective group and purification.

Further in the compounds which contain at the 5'-terminal thereof, for example, an alkyl-substituted thiophosphoryl group, an alkyl-substituted phosphoryl group or an alkyl-substituted alkylaminophosphoryl group, the chain elongation of the oligomer is effected on the polymer support by the process described

above; after completion of the oxidation, the oxidation product is condensed with an alkyl-H-phosphonate and the condensation product is oxidized with sulfur ( $S_8$ ), iodine ( $I_2$ ) or an alkylamine/carbon tetrachloride followed by removal of the protective group and purification. The derivatives in which cholesterol is bound at the 5'-terminal via a spacer can be likewise obtained by condensing with mono(monomethoxytritylated) alkanediol H-phosphonate, treating with an acid, condensing with cholesteryl-H-phosphonate, oxidizing, removing the protective group and purification.

On the other hand, the compounds containing, for example, a hydroxyalkylphosphoryloxy group or a hydroxyalkylthiophosphoryloxy group at the 3'-terminal thereof can be obtained by condensing, oxidizing, removing the protective group and purification in a similar manner, using as a starting material a polymer support having bound thereto an alkanediol monosuccinate.

The anti-AIDS viral activity can be determined by the method described in a publication (cf., Harada, S., et al., Science, 229, 563-566, 1985), using HTLV-III $\beta$  (cf., Popovic, M., et al., Science, 224, 497-500, 1984) which is an isolated strain of AIDS virus. According to this method, MT-4 cells or HTLV-I positive cell line is used as the target cell so that this method is highly sensitive to viral infection and can exhibit the cytopathic effect highly efficiently. Using this method, the cytopathogen inhibitory effect was examined with Compounds VI, VII, VIII, IX, X, XI, XII, XV, XVI, XVII, XVIII, XXIII and XXIV, after each compound was diluted with medium into various concentrations. As the result, the cytopathogen inhibitory effect and the effect of inhibiting development of viral specific antigen positive cells were noted with Compound VI in a concentration of 10  $\mu$ M, Compounds VII, X and XII in a concentration of 15  $\mu$ M, and Compound VIII in a concentration of 1  $\mu$ M. No cytotoxicity was detected even in the maximum concentration of 120  $\mu$ M tested. On the other hand, the cytopathogen inhibitory effect and the effect of inhibiting development of viral specific antigen positive cells were noted with Compound IX in a concentration of 240  $\mu$ M and Compound XI in a concentration of 30  $\mu$ M. No cytotoxicity was detected in these concentrations.

Similarly, these inhibitory effects were noted with Compound XVI in a concentration of 1  $\mu$ M. On the other hand, these inhibitory effects were noted with Compound XV in a concentration of 7.5  $\mu$ M, Compound XVII in a concentration of 15  $\mu$ M and Compound XVIII in a concentration of 120  $\mu$ M. No cytotoxicity was detected with these compounds even in the maximum concentrations (60  $\mu$ M with Compounds XV and XVI and 120  $\mu$ M with Compounds XVII and XVIII).

Further Compound XXIII substantially completely inhibited cytopathogen in a concentration of 3.8  $\mu$ M and appearance of viral specific antigen positive cells in a concentration of 7.5  $\mu$ M, respectively. Compound XXIV substantially completely inhibited cytopathogen in a concentration of 7.5  $\mu$ M and appearance of viral specific antigen positive cells in a concentration of 30  $\mu$ M, respectively. No cytotoxicity was detected with these compounds even in the maximum concentrations (60  $\mu$ M).

On the other hand, no cytopathogen inhibitory effect was recognized with deoxyoligocytidine phosphorothioate (15 mer) or deoxyoligonucleotide (phosphate type) having the same nucleotide sequence as in Compound VI or XI in a concentration of 10  $\mu$ M.

As is clear from the foregoing, the novel 2'-O-methylribooligonucleotide derivatives and novel oligoribonucleotide derivatives in accordance with the present invention have the anti-AIDS viral activity and are expected to use as anti-AIDS drugs.

The antiviral agent of the present invention may be used in a range of 0.01 to 2,000 mg, preferably 0.02 to 500 mg.

The effective component may be used in the form of, for example, a capsule, an elixir, a microcapsule or a suspension.

The compound used as the effective component of the present invention can be administered to the patient who requires treatment or prophylaxis, in a dose of 0.01 to 1,000 mg per person, generally several times in a daily dose of 0.02 to 2,000 mg. The dose can be varied depending upon condition of disease, body weight of the patient and other factors recognized by one skilled in the art.

The compound used in the present invention or physiologically acceptable salt compound or a mixture thereof can be mixed in an amount of approximately 0.2 to 500 mg together with vehicles, carriers, diluents, binders, antiseptics, stabilizers, flavors, etc. in a unit dose form required to make pharmaceutical preparations generally admitted. An amount of the active substance in these compositions or preparations is incorporated to give an appropriate dose in a range indicated.

Specific examples of drugs which can be incorporated into tablets, capsules, etc. are given below: binders such as tragacanth, gum arabic, corn starch or gelatin; excipients such as fine crystalline cellulose; swelling agents such as corn starch, pregelatinized starch, alginic acid, etc.; lubricants such as magnesium stearate; sweeteners such as sucrose, lactose or saccharine; flavors such as peppermint, Gaultheria adenothrix oil or cherry, etc. In case that the preparation unit form is a capsule, a liquid carrier such as oils and fats can additionally be incorporated into the materials of type described above. A variety of other

materials may be incorporated as protectives or in order to change physical form of the preparation unit by another method. For example, tablets can be coated with shellac or sugar or with both of them. Syrup or elixir may contain sucrose as a sweetener, methyl- and propylparabens as antiseptics and pigments and flavors such as cherry or orange flavor.

5 In the case of enteric coating, for example, 8% aqueous solution of hydroxyphenylmethyl cellulose is used as a coating. pretreatment agent, 10% aqueous solution of hydroxypropylmethyl cellulose phthalate and 3% aqueous solution of polyacetyne are used as coating agents. They are used respectively to make enteric coating preparations in a conventional manner.

10 A sterile composition for injection can be formulated in a conventional manner to prepare pharmaceutical preparations by dissolving or suspending the active substance, naturally occurring plant oils such as sesame oil, coconut oil, peanut oil, cotton seed oil, etc. or synthetic fatty vehicle such as ethyl oleate in a vehicle such as water for injection. Buffer agents, antiseptics, antioxidants, etc. may be added, if necessary.

## 15 Examples

Hereafter the present invention is concretely described by referring to the examples below.

### 20 Example 1 Production of N<sup>4</sup>-benzoyl-5'-O-dimethoxytrityl-2'-O-methylcytidine-3'-O-(H-phosphonate) (Compound I):

To 50 ml of a solution of 436  $\mu$ l (5 mmols) of phosphorus trichloride and 5.5 ml (50 mmols) of N-methylmorpholine in dichloromethane was added 1.197 g (17.3 mmols) of 1,2,4-triazole. The mixture was  
25 stirred at room temperature for 30 minutes. To the mixture was added 17 ml of a solution of 664.7 mg (1 mmol) of N<sup>4</sup>-benzoyl-5'-O-dimethoxytrityl-2'-O-methylcytidine in dichloromethane over 10 minutes. The mixture was further stirred at room temperature, whereby the progress of the reaction was monitored by thin layer chromatography (mobile phase: dichloromethane/2% triethylamine/8% methanol) using silica gel 60. After it was confirmed that the raw material spot (Rf value: 0.60) disappeared and a new spot derived  
30 from Compound I appeared at Rf value of 0.41, 40 ml of triethylamine-bicarbonate buffer (1 M, pH 7.5) was added. The aqueous layer was extracted with 15 ml of dichloromethane. The dichloromethane layers were combined, dried over anhydrous sodium sulfate and then evaporated to dryness. Next, the resulting foamy residue was dissolved in 3 ml of dichloromethane containing 2% triethylamine and the solution was purified by passing through a column packed with 24 g of silica gel 60. That is, elution was carried out using 100 ml  
35 of dichloromethane/2% triethylamine, then 200 ml of dichloromethane/2% triethylamine/3% methanol and further 200 ml of dichloromethane/2% triethylamine as the mobile phase and the pure fraction of Compound I obtained was evaporated to dryness. The resulting residue was dissolved in 40 ml of dichloromethane and the solution was washed with 15 ml of 1 M triethylamine-bicarbonate buffer (pH 7.5). After drying over anhydrous sodium sulfate, the dichloromethane layer was evaporated to dryness to give 703 mg (0.849  
40 mmol) of Compound I in yield of 84.9%.

In a manner similar to the operation described above, N<sup>6</sup>-benzoyl-5'-O-dimethoxytrityl-2'-O-methyladenosine-3'-O-(H-phosphonate) (Compound II), N<sup>2</sup>-isobutyl-5'-O-monomethoxytrityl-2'-O-methylguanosine-3'-O-(H-phosphonate) (Compound III), 5'-O-dimethoxytrityl-2'-O-methyluridine-3'-O-(H-phosphonate) (Compound IV), 5'-O-dimethoxytrityl-2'-O-methylinosine-3'-O-(H-phosphonate) (Compound V)  
45 were obtained in yields of 93.9%, 49.4%, 77.4% and 56.0%, respectively.

Next, <sup>31</sup>P-NMR of these compounds was measured. With respect to Compounds I, II and III, a part of each compound was dissolved in dichloromethane and after mixing the solution with an equimolar amount of 1,8-diazabicyclo[5.4.0]undec-7-ene (simply referred to as DBU), the mixture was dropped into n-hexane to form the DBU salt, the salt was ground into powders and the powders were measured. On the other  
50 hand, Compounds IV and V were measured as they were, i.e., as the triethylamine salts. As a solvent for the measurement, d<sub>5</sub> pyridine was used. D<sub>2</sub>O solution of phosphoric acid was used as the external standard.

Their chemical shifts (ppm) were as follows.

|              |           |
|--------------|-----------|
| Compound I   | -1.79 ppm |
| Compound II  | -2.20 ppm |
| Compound III | -2.04 ppm |
| Compound IV  | -0.73 ppm |
| Compound V   | -0.40 ppm |

5

10 Example 2 Production of 5'-O-dimethoxytrityl-2'-O-(tert-butyldimethylsilyl)inosine-3'-O-(H-phosphonate) (Compound XXI) and 5'-O-dimethoxytrityl-3'-O-(tert-butyldimethylsilyl)inosine 2'-O-(H-phosphonate) (Compound XXII)

5'-O-Dimethoxytritylinosine, 28.5 g (50 mmols), synthesized in a conventional manner was dissolved in  
 15 100 ml of pyridine and 9.5 g (140 mmols) of imidazole and 9.9 g (66 mmols) of tert-butyldimethylchlorosilane were added to the solution. The mixture was stirred at room temperature. Five hours after, the progress of the reaction was monitored by thin layer chromatography (mobile phase: chloroform/methanol = 10 : 1) using silica gel 60. After it was confirmed that the raw material spot (Rf value: 0.08) disappeared and new spots of 5'-O-dimethoxytrityl-2'-O-(tert-butyldimethylsilyl)inosine  
 20 (Compound XIX) and 5'-O-dimethoxytrityl-3'-O-(tert-butyldimethylsilyl)inosine (Compound XX) newly appeared at Rf values of 0.44 and 0.33, respectively, 150 ml of water was added under chilling with ice water. The mixture was extracted once with 350 ml and twice with 100 ml of dichloromethane. The dichloromethane layers were combined, washed twice with 150 ml water and then concentrated. After the resulting oily residue was co-evaporated together with toluene, it was dissolved in 50 ml of dichloromethane  
 25 and the solution was purified by passing through a column packed with 350 g of silica gel 60. That is, elution was performed using 7 liters of dichloromethane in which an acetone concentration was stepwise increased from 2% (v/v) to 13% (v/v) as the mobile phase. Pure fractions of Compound XIX eluted in acetone concentrations of 2% to 5% were collected and evaporated to dryness (4.05 g, 5.89 mmols). Next, the mixture of Compound XIX and Compound XX eluted in acetone concentrations of 6 to 13% was  
 30 evaporated to dryness. The residue was again purified by passing through a column packed with 350 g of silica gel 60. That is, elution was performed using as the mobile phase 6 liters of dichloromethane in which an acetone concentration was stepwise increased from 1% (v/v) to 12% (v/v) and then in acetone concentrations of 7% to 12% using 2.5 liters of ethyl acetate to give the pure fraction of Compound XIX. The fractions of a mixture of Compound XIX and Compound XX eluted with ethyl acetate and the pure  
 35 fraction of Compound XX were evaporated to dryness and dried under reduced pressure, respectively. Yielded amount of Compound XIX was 4.3 g, 6.26 mmols; when combined with the isolate of first column chromatography, 8.35 g, 12.15 mmols, showing yield of 24.3%. The mixture of Compound XIX and Compound XX was obtained in an amount of 1.13 g, 1.65 mmols in yield of 3.3% and, Compound XX was obtained in an amount of 2.66 g, 3.88 mmols, in yield of 7.8%.

40 Compound XIX: FAB mass spectrum, 685 (M-H<sup>+</sup>);

<sup>1</sup>H-NMR spectrum (CDCl<sub>3</sub>, δ ppm)

8.03 (s, H<sub>8</sub> or H<sub>2</sub>), 7.99 (s, 1, H<sub>2</sub> or H<sub>8</sub>), 7.45-6.81 (m, 13, Ar-H), 6.01 (d, 1, H<sub>1</sub>'), 4.89 (m, 1, H<sub>2</sub>'), 4.33 (m, 1, H<sub>3</sub>'), 4.27 (m, 1, H<sub>4</sub>'), 3.79 (s, 6, MeO), 3.45 (m, 2, H<sub>5</sub>'), 2.71 (d, 1, OH<sub>3</sub>'), 0.85 (s, 9, tert-Bu-Si), 0.10 (s, 3, Me-Si), -0.125 (s, 3, Me-Si);

45 UV absorption spectrum (ethanol) λ<sub>max</sub> = 247nm, λ<sub>min</sub> = 223nm, λ<sub>270</sub> nm-shoulder.

Compound XX : FAB mass spectrum, 685 (M-H<sup>+</sup>);

<sup>1</sup>H-NMR spectrum (CDCl<sub>3</sub>, δ ppm)

8.11 (s, H<sub>8</sub> or H<sub>2</sub>), 8.05 (s, 1, H<sub>2</sub> or H<sub>8</sub>), 7.44-6.80 (m, 13, Ar-H), 5.98 (d, 1, H<sub>1</sub>'), 4.62 (m, 1, H<sub>2</sub>'), 4.50 (m, 1, H<sub>3</sub>'), 4.18 (m, 1, H<sub>4</sub>'), 3.77 (s, 6, MeO), 3.38 (m, 2, H<sub>5</sub>'), 3.07 (d, 1, OH<sub>2</sub>'), 0.89 (s, 9, tert-Bu-Si), 0.09 (s, 3, Me-Si), 0.02 (s, 3, Me-Si);

50 UV absorption spectrum (ethanol) λ<sub>max</sub> = 247nm, λ<sub>min</sub> = 223nm, λ<sub>270</sub> nm-shoulder.

Proton of the sugar moiety in the <sup>1</sup>H-NMR spectrum was identified by the spin decoupling method.

55 Device for measurement:

FAB mass spectrum: JEOL JMS-DX300 (manufactured by JEOL, Ltd )

<sup>1</sup>H-NMR spectrum: Varian XL-300, 300 MHz

UV absorption spectrum: Hitachi 320 Spectrophotometer

Next, 0.872 ml (10 mmols) of phosphorus trichloride was added to a solution of 11 ml (100 mmols) of N-methylmorpholine and 2.38 g (34.5 mmols) of 1,2,4-triazole in dichloromethane. The mixture was stirred at room temperature for 30 minutes. To the liquid mixture was added 32 ml of a solution of 1.37 g (2 mmols) of 5'-O-dimethoxytrityl-2'-O-(tert-butyldimethylsilyl)-inosine (Compound XIX) in dichloromethane-dimethylformamide (15 : 1, v/v) over 10 minutes. After stirring at room temperature for further 20 minutes, the progress of the reaction was monitored by thin layer chromatography (mobile phase: dichloromethane/2% triethylamine/8% methanol) using silica gel 60. After it was confirmed that the raw material spot (R<sub>f</sub> value: 0.64) disappeared and a spot derived from 5'-O-dimethoxytrityl-2'-O-(tert-butyldimethylsilyl)inosine-3'-O-(H-phosphonate) (Compound XXI) appeared at R<sub>f</sub> value of 0.44, 80 ml of 1M triethylamine-bicarbonate buffer (pH 7.5) was added. The aqueous layer was extracted with 40 ml of dichloromethane. The dichloromethane layers were combined, dried over anhydrous sodium sulfate and then evaporated to dryness. The procedure up to this stage was repeated once again on the same scale. The resulting foamy residues obtained by both procedures were combined and dissolved in 10 ml of dichloromethane containing 2% triethylamine. The solution was purified by passing through a column packed with 40 g of silica gel 60. That is, using as the mobile phase, in sequence, 150 ml of dichloromethane/2% triethylamine, 200 ml of dichloromethane/2% triethylamine/2% methanol, 200 ml of dichloromethane/2% triethylamine/4% methanol, 200 ml of dichloromethane/2% triethylamine/6% methanol, 200 ml of dichloromethane/2% triethylamine/8% methanol and 100 ml of dichloromethane/2% triethylamine/9% methanol, elution was carried out and the pure fraction of Compound XXI thus obtained was evaporated to dryness. The resulting residue was dissolved in 50 ml of dichloromethane and the solution was washed with 40 ml of 1 M triethylamine-bicarbonate buffer (pH 7.5). After drying over anhydrous sodium sulfate, the dichloromethane layer was evaporated to dryness to give 3.0 g (3.53 mmols) of Compound XXI in yield of 88%.

5'-O-Dimethoxytrityl-3'-O-(tert-butyldimethylsilyl)inosine-2'-O-(H-phosphonate) (Compound XXII) was isolated in a manner similar to above in yield of 72.5%.

Compound XXI: FAB mass spectrum, 749 (M-H<sup>+</sup>);

<sup>32</sup>P-NMR spectrum (d<sub>5</sub>-pyridine, δ ppm, external standard 5% H<sub>3</sub>PO<sub>4</sub> in D<sub>2</sub>O):

-1.469, <sup>1</sup>J<sub>P-H</sub> = 608.7Hz, <sup>3</sup>J<sub>P-H</sub> = 10.2Hz;

<sup>1</sup>H-NMR spectrum (CDCl<sub>3</sub>, δ ppm)

8.40 (s, H<sub>8</sub> or H<sub>2</sub>), 8.00 (s, 1, H<sub>2</sub> or H<sub>8</sub>), 7.44-7.18 (m, 13, Ar-H), 7.07 (d, 1, P-H, J=625Hz), 6.32 (d, 1, H<sub>1</sub>'), 4.82 (m, 1, H<sub>2</sub>'), 4.85 (m, 1, H<sub>3</sub>'), 4.46 (m, 1, H<sub>4</sub>'), 3.78 (s, 6, MeO), 3.47 (m, 2, H<sub>5</sub>'), 0.72 (s, 9, tert Bu-Si), 0.01 (s, 3, Me-Si), -0.21 (s, 3, Me-Si).

Compound XXII: FAB mass spectrum, 749 (M-H<sup>+</sup>);

<sup>32</sup>P-NMR spectrum (d<sub>5</sub>-pyridine, δ ppm, external standard 5% H<sub>3</sub>PO<sub>4</sub> in D<sub>2</sub>O):

-1.858, <sup>1</sup>J<sub>P-H</sub> = 612.8Hz, <sup>3</sup>J<sub>P-H</sub> = 10.7Hz;

<sup>1</sup>H-NMR spectrum (CDCl<sub>3</sub>, δ ppm)

7.98 (s, H<sub>8</sub> or H<sub>2</sub>), 7.93 (s, 1, H<sub>2</sub> or H<sub>8</sub>), 7.41-6.77 (m, 13, Ar-H), 6.95 (d, 1, P-H, J=634Hz), 6.23 (d, 1, H<sub>1</sub>'), 5.31 (m, 1, H<sub>2</sub>'), 4.50 (m, 1, H<sub>3</sub>'), 4.23 (m, 1, H<sub>4</sub>'), 3.76 (s, 6, MeO), 3.31 (m, 2, H<sub>5</sub>'), 0.86 (s, 9, tert-Bu-Si), 0.14 (s, 3, Me-Si), 0.03 (s, 3, Me-Si).

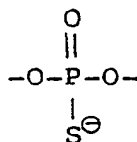
Example 3 Production of 5'-O-monomethoxytrityl-N<sup>2</sup>-isobutyllyl 3'-O-methylguanosine-bound controlled pore glass:

5'-O-monomethoxytrityl N<sup>2</sup>-isobutyllyl 3'-O-methylguanosine synthesized in a conventional manner was led to 2'-O-succinylpentachlorophenyl ester by a known process (cf., Miyoshi, K., et al., Nucleic Acids Res., 8, 5491, 1980). That is, 639 mg (1 mmol) of 5'-O-monomethoxytrityl-N<sup>2</sup>-isobutyllyl-3'-O-methylguanosine and 183 mg of 4-N,N-dimethylaminopyridine (merely referred to as DMAP) were dissolved in 4 ml of absolute pyridine. While stirring at room temperature, 150 mg (1.5 mmol) of succinic anhydride was added to the solution. After stirring for 90 minutes, the reaction liquid was distilled under reduced pressure. The resulting oily residue was dissolved in 35 ml of chloroform. The solution was washed 3 times with 30 ml of 0.1 M triethylamine-bicarbonate buffer (pH 7.5). After drying over anhydrous sodium sulfate, the chloroform layer was evaporated to dryness under reduced pressure. The obtained residue was dissolved in 5 ml of dimethylformamide (DMF) and 400 mg (1.5 mmol) of pentachlorophenol and 309 mg (1.5 mmol) of dicyclohexylcarbodiimide were added to the solution followed by stirring at room temperature for 13.5 hours. The precipitated insoluble matters were removed by filtration and the filtrate was removed by distillation under reduced pressure. The resulting residue was dissolved in 3 ml of chloroform and the

solution was purified by column chromatography using a column packed with 20 g of silica gel 60. That is, elution was carried out using as the mobile phase, in sequence, 100 ml of chloroform, 100 ml of chloroform/0.3% methanol, 100 ml of chloroform/0.5% methanol and further 100 ml of chloroform/0.6% methanol and the resulting pure fractions of 5'-O-monomethoxytrityl-N<sup>2</sup>-isobutyl 3'-O-methylguanosine-2'-O-succinyl-pentachlorophenyl ester were collected and evaporated to dryness under reduced pressure to give 530 mg of the ester in yield of 53.6% (R<sub>f</sub> value: 0.73 when developed with chloroform : methanol = 20 : 1 in silica gel 60 thin layer chromatography). Next, the whole amount (530 mg) of the ester was dissolved in 5.4 ml of DMF; on the other hand, 1 g of controlled pore glass (CPG/long chain alkylamine, pore diameter: 500 Å, particle size: 122-177 μ, NH<sub>2</sub>: 30 μmols/g, manufactured by Pierce Chemical Inc.) was charged in a reactor equipped with a glass filter and washed with 1 ml of DMF followed by drying for a minute in an argon gas flow. The aforesaid pentachlorophenyl ester DMF solution, 5.4 ml, and 73 μl of triethylamine were added to the pore glass, respectively. After sealing, the reaction was carried out at 30 °C overnight. The reaction liquid was filtered under an argon pressure. After washing 3 times with 5 ml of DMF and then 3 times with 5 ml of tetrahydrofuran (THF), the controlled pore glass was dried for a minute in an argon flow. Next, a liquid mixture of 410 mg of DMAP, 1.4 g of 2,6-lutidine, 660 μl of acetic anhydride and 6 ml of THF was added to the pore glass. The mixture was shaken at 30 °C for 15 minutes to acetylate the unreacted amino group. After filtration, the reaction mixture was washed 3 times with 5 ml of THF and then 3 times with 5 ml of diethyl ether and dried in an argon flow to give 1 g of 5'-O-monomethoxytrityl-N<sup>2</sup>-isobutyl-3'-O-methyl-guanosine-bound controlled pore glass. A small amount of the product was weighed and the monomethoxytrityl group was cleaved with 3% trichloroacetic acid. The isolated tritanol was subjected to quantitative colorimetry with a perchloric acid-ethanol mixture. As the result, the bound amount was 26 μmols/g.

Furthermore, 5'-O-dimethoxytrityl-N<sup>6</sup>-benzoyl-3'-O-methyladenosine-bound controlled pore glass, 5'-O-dimethoxytrityl-3'-O-methylinosine-bound controlled pore glass, 5'-O-dimethoxytrityl-3'-O-methyluridine bound controlled pore glass, 5'-O-dimethoxytrityl-N<sup>4</sup>-benzoyl 3'-O-methylcytidine-bound controlled pore glass, 5'-O-dimethoxytrityl-2'-O-(tert-butyl dimethylsilyl)inosine-bound controlled pore glass and monomethoxy tritylhexanediol-bound controlled pore glass were produced, respectively, in a manner similar to the above.

Example 4 Production of 5' Amp Cmp Amp Cmp Cmp Cmp Amp Amp Ump Ump Cmp Ump Gmp Amp Amp Amp Amp Ump Gmp Gm 3' (Compound VI), wherein m represents 2'-O-methylnucleoside unit; m' represents 3'-O-methylnucleoside unit; and P represents:



N<sup>2</sup>-Isobutyl-5'-O-monomethoxytrityl-3'-O-methylguanosine-bound polymer support, 40 mg (binding amount: 1.0 μmol), was packed in a cartridge and the cartridge was set in a DNA synthesizer (model 380A, manufactured by Applied Biosystems Inc.). Following the procedure shown in Table 1, 2'-O-methyl-ribonucleoside-3'-O-(H phosphonate) compounds (Compounds I through IV) were treated using III, IV, II, II, II, II, III, IV, I, IV, IV, II, II, I, I, I, II, I and II in this order. After repeating 19 cycles, the intermediate for preparing the polymer support was withdrawn from the cartridge and shifted to a reactor equipped with a glass filter. After washing with 1 ml of acetonitrile, the intermediate was dried in an argon gas flow. Next, 2 ml of a solution of 0.2 M sulfur (S<sub>8</sub>) in triethylamine : carbon disulfide : pyridine (1 : 12 : 12, volume ratio) was added to the intermediate. After shaking, the mixture was allowed to stand for 12 hours. The reaction mixture was filtered and the intermediate for synthesizing the polymer support was washed 5 times with 2 ml of carbon disulfide and once with 2 ml of diethyl ether. After drying in an argon flow, the intermediate was transferred to a glass-made bial of a 3 ml volume. 28% Ammonia water, 2 ml, was charged in the bial and sealed followed by heating at 55 °C for 5 hours. The polymer support was removed by filtration and the reaction liquid was evaporated to dryness under reduced pressure. The resulting residue was dissolved in 300 μl of 0.1 M triethyl ammonium-acetic acid buffer (pH 7.0, hereafter simply referred to as TEAA) containing 10% acetonitrile. The solution was purified through a column having a diameter of 1 cm and a

length of 12 cm, packed with reverse phase silica gel (Waters Co., Ltd., Prep PAK-500/C-18). That is, 0.1 M TEAA buffer (pH 7.0) having a linear gradient of 10% to 35% acetonitrile was used as the mobile phase and quantitative determination was performed at absorbancy at a wavelength of 254 nm to give the fraction of Compound VI having a dimethoxytrityl group at the 5'-terminal. After the solvent was distilled off under reduced pressure, the residue was co-evaporated together with 2 ml of water 3 times and 3 ml of 80% acetic acid was added thereto followed by stirring for 10 minutes. After the reaction liquid was evaporated to dryness, the residue was further co-evaporated together with water to remove acetic acid. To the residue was added 1.5 ml of 0.1 M triethylamine-bicarbonate buffer (pH 7.5) containing 10% acetonitrile. The mixture was washed twice with 1.5 ml of diethyl ether. The aqueous layer was evaporated to dryness and 200  $\mu$ l of 0.1 M TEAA buffer (pH 7.0) containing 10% acetonitrile was added to the resulting residue. After passing through a 0.45  $\mu$  filter (manufactured by Millipore Corp.), purification was performed by high performance liquid chromatography. That is, YMC Pack ODS (manufactured by Yamamura Science Co., Ltd.) column was used and 0.1 M TEAA buffer (pH 7.0) having a linear gradient of 10% to 70% acetonitrile was used as the mobile phase. The main peak eluted at a flow rate of 1.2 ml/min was fractionated to give 52.9 OD<sup>260 nm</sup> units (about 226 nmols) of Compound VI in yield of 22.6%.

Compound VI was dissolved in D<sub>2</sub>O containing 10 mM triethylamine-bicarbonate buffer (pH 7.5) and <sup>31</sup>P-NMR was measured (device for measurement: JEOL JMS-DX300, manufactured by JEOL Inc.). When trimethyl phosphate was used as the external standard, Compound VI showed a characteristic signal in multiplet phosphorothioate at 51-55 ppm.

Table 1

| One Cycle of Procedure for Chain Elongation Reaction |   |                              |                |
|--|---|------------------------------|----------------|
| Operation Number                                     | Name of Reagent or Operation  | Liquid Feeding Time (second) | Number of Time |
| 1.   | Washing with acetonitrile   | 30                           | 1              |
| 2.   | Drying in argon   | 20                           | 1              |
| 3.   | 3% Trichloroacetic acid/dichloromethane   | 30                           | 6              |
| 4.   | Drying in argon   | 5                            | 1              |
| 5.   | Washing with acetonitrile   | 30 + 60                      | 2              |
|  | Drying in argon   | 5 + 20                       |                |
| 6.   | 0.2 M Compounds I-V, XV-XIII, XXI or XXII/acetonitrile 1*0 M Pivaloyl chloride/pyridine | 13                           | 1              |
| 7.   | Allowing to stand   | 180                          | 1              |
| 8.   | Drying in argon   | 15                           | 1              |
| 9.   | Washing with acetonitrile   | 30                           | 3              |
|  | Drying in argon   | 20                           |                |



Furthermore using 2  $\mu$ moles of nucleoside-bound support as the starting material in a manner similar to the procedure described above, Compound VII was obtained in 26 OD<sup>260 nm</sup> units (about 132 nmols) in yield of about 6.3% using, in sequence, III, IV, II, II, II, II, III, IV, I, VI, IV, II, II, I, I and I in the condensation cycle; Compound VIII was obtained in 41.5 OD<sup>260 nm</sup> units (about 136 nmols) in yield of about 6.2%, using, in sequence, II, II, IV, II, III, III, IV, II, II, II, II, III, IV, I, IV, IV, II, II, I, I, I, I, II, I and II in the condensation cycle; Compound IX was obtained in 62 OD<sup>260 nm</sup> units (about 528 nmols) in yield of about 24.2%, using, in sequence, II, III, IV, I, IV, IV, II, II and I in the condensation cycle; and Compound X was obtained in 37 OD<sup>260 nm</sup> units (about 180 nmols) in yield of about 8.7%, using, in sequence, IV, I, II, I, I, III, I, III, III, III, I, IV, IV, III, IV, I, I, IV and III in the condensation cycle. Furthermore, using 1  $\mu$ mol of nucleoside-bound support as the starting material, Compound XI was obtained in 16.0 OD<sup>260 nm</sup> units (about 72 nmols) in yield of about 7.2%, using, in sequence, IV, I, IV, II, III, II, I, IV, I, III, III, II, I, I, I, IV, I, III, II and III in the condensation cycle.

Furthermore, in a manner similar to the procedure described above, Compound XV was obtained in 25 OD<sup>260 nm</sup> units (about 81.7 nmols) showing yield of about 3.4%, using as the starting material 2.4  $\mu$ moles of 5'-O-dimethoxytrityl-N<sup>6</sup>-benzoyl-3'-O-methyladenosine-bound controlled pore glass and using Compound II by 19 cycles in the condensation cycle; Compound XVI was obtained in 74 OD<sup>260 nm</sup> units (about 301 nmols) showing yield of about 8.3%, using as the starting material 3.6  $\mu$ moles of 5'-O-dimethoxytrityl 3'-O-methylinosine-bound polymer support and using Compound V by 19 cycles in the condensation cycle; Compound XVII was obtained in 58 OD<sup>260 nm</sup> units (about 292 nmols) showing yield of about 8.1%, using as the starting material 3.6  $\mu$ moles of 5'-O-dimethoxytrityl-3'-O-methyluridine-bound polymer support and using Compound IV by 19 cycles in the condensation cycle; and Compound XVIII was obtained in 25 OD<sup>260 nm</sup> units (about 168 nmols) showing yield of about 4.7%, using as the starting material 3.6  $\mu$ moles of 5'-O-dimethoxytrityl-N<sup>4</sup>-benzoyl-3'-O-methylcytidine-bound polymer support and using Compound I by 19 cycles in the condensation cycle; respectively.

In a similar manner, Compounds XXVII, XXVIII and XXIX were produced.

#### Example 5 Production of Compound XII

In a reactor equipped with a glass filter was charged 120 mg (total binding amount, 3.12  $\mu$ moles) of polymer support having bound thereto N<sup>2</sup>-isobutyl-5'-O-monomethoxytrityl 3'-O-methylguanosine. Following the procedure shown in Table 1, 2'-O-methylribonucleoside 3'-O-(H-phosphonate) compounds (Compounds I to IV) were used in the order of III, IV, II, II and II, which was repeated by 5 cycles. However, 2 ml each was used in operation Nos. 1, 3, 5, 6 and 9. Next, 2 ml of a solution of 0.2 M sulfur (S<sub>8</sub>) in triethylamine : carbon disulfide : pyridine (1 : 12 : 12, volume ratio) was added to the system. After shaking, the mixture was allowed to stand for 70 minutes. The reaction mixture was filtered and the intermediate for synthesizing the polymer support was washed 5 times with 2 ml of carbon disulfide and 5 times with 2 ml of acetonitrile followed by drying in an argon flow. Furthermore, the procedure was repeated by 9 cycles as shown in Table 1, using Compounds II, III, IV, I, IV, IV, II, II and I, in this order. Thereafter, 2 ml of a solution of 0.1 M iodine (I<sub>2</sub>) in pyridine : N-methylimidazole : H<sub>2</sub>O : THF (5 : 1 : 5 : 90, volume ratio) was added to the system. The mixture was allowed to stand at room temperature for 20 minutes. The reaction liquid was filtered and 2 ml of a solution of 0.1 M iodine (I<sub>2</sub>) in triethylamine : H<sub>2</sub>O : THF (5 : 5 : 90, volume ratio) was then added to the system. The allowing to stand at room temperature for 20 minutes, the reaction liquid was filtered. Furthermore, the procedure was repeated by 5 cycles as shown in Table 1, using Compounds I, I, II, I and II, in this order followed by the same sulfur oxidation treatment as described above overnight.

The reaction liquid was filtered and the intermediate for synthesizing polymer support was washed 5 times with 2 ml of carbon disulfide and once with 2 ml of diethyl ether. After drying in an argon flow, the intermediate was transferred to a glass-made bial of a 3 ml volume. 28% Ammonia water, 2 ml, was charged in the bial and sealed followed by heating at 55 °C for 5 hours. The polymer support was removed by filtration and the reaction liquid was evaporated to dryness under reduced pressure. The resulting residue was dissolved in 300  $\mu$ l of 0.1 M triethyl ammonium-acetic acid buffer (pH 7.0, hereafter simply referred to as TEAA) containing 10% acetonitrile. The solution was purified through a column having a diameter of 1 cm and a length of 12 cm, packed with reverse phase silica gel (Waters Co., Ltd., Prep PAK-500/C-18). That is, 0.1 M TEAA buffer (pH 7.0) having a linear gradient of 10% to 35% acetonitrile was used as the mobile phase and quantitative determination was performed at absorbancy at a wavelength of 254 nm to give the fraction of Compound XII having a dimethoxytrityl group at the 5'-terminal. After the solvent was distilled off under reduced pressure, the residue was co-evaporated together with 2 ml of water 3 times

and 3 ml of 80% acetic acid was added thereto followed by stirring them for 10 minutes. After the reaction liquid was evaporated to dryness, the residue was further co-evaporated together with water to remove acetic acid. To the residue was added 1.5 ml of 0.1 M triethylamine-bicarbonate buffer (pH 7.5) containing 10% acetonitrile. The mixture was washed twice with 1.5 ml of diethyl ether. The aqueous layer was  
 5 evaporated to dryness and 200  $\mu$ l of 0.1 M TEAA buffer (pH 7.0) containing 10% acetonitrile was added to the resulting residue. After passing through a 0.45  $\mu$  filter (manufactured by Millipore Corp.), purification was performed by high performance liquid chromatography. That is, YMC Pack ODS (manufactured by Yamamura Science Co., Ltd.) column was used and 0.1 M TEAA buffer (pH 7.0) having a linear gradient of 10% to 50% acetonitrile was used as the mobile phase. The main peak eluted at a flow rate of 1.2 ml/min  
 10 was fractionated to give 65.2 OD<sup>260 nm</sup> units (about 278 nmols) of Compound XII in yield of 8.9%.

Compound XII was dissolved in D<sub>2</sub>O containing 10 mM triethylamine-bicarbonate buffer (pH 7.5) and <sup>31</sup>P-NMR was measured (device for measurement: JEOL JMS-DX300, manufactured by JEOL Inc.). When trimethyl phosphate was used as the external standard, Compound XII gave a characteristic signal in multiplet phosphorothioate at 51-55 ppm and a singlet signal derived from phosphate at -4.2 ppm in an  
 15 integration ratio of 7 : 12. From the results of this NMR and the order for the oxidation at the chain elongation step, it has been made clear that Compound XII contains 5 phosphorus atoms from the 5'-terminal and 5 phosphorus atoms from the 3'-terminal, among which 2 phosphorus atoms in average are thiophosphates and the balance has phosphate diester bonds.

#### 20 Example 6 Production of Compound XXIII

5'-O-Dimethoxytrityl-2'-O-(tert-butyldimethylsilyl)inosine-CPG (controlled pore glass), 40 mg (binding amount: 1.0  $\mu$ mol) each, was packed in 3 cartridges and the cartridges were set in a DNA synthesizer  
 25 (model 380A, manufactured by Applied Biosystems Inc.). Following the procedure shown in Table 1, 5'-O-dimethoxytrityl-2'-O-(tert-butyldimethylsilyl)inosine-3'-O-(H-phosphonate) (Compound XXI) was used together with pivaloyl chloride. After repeating 19 cycles, the intermediate for preparing the polymer support was withdrawn from each cartridge and shifted to a reactor equipped with a glass filter. After washing with 1 ml of acetonitrile, the intermediate was dried in an argon gas flow. Next, 2 ml of a solution of 0.2 M sulfur (S<sub>8</sub>)  
 30 in triethylamine : carbon disulfide : pyridine (1 : 12 : 12, volume ratio) was added to the intermediate. After shaking, the mixture was allowed to stand for 2 hours. The reaction liquid was filtered and the intermediate for synthesizing the polymer support was washed 5 times with 2 ml of carbon disulfide and once with 2 ml of diethyl ether. After drying in an argon flow, the intermediate was transferred to a glass-made bial of a 3 ml volume. 28% Ammonia water, 1.5 ml, and 0.5 ml of ethanol were charged in the bial and sealed followed  
 35 by heating at 55 °C for 5 hours. The polymer support was removed by filtration and washed with 1 ml of ethanol and then with 1 ml of 50% ethanolic water. The filtrates were combined and evaporated to dryness. To the resulting residue was added 1.5 ml of THF solution (1 M) of tetra-(n-butyl)ammonium fluoride. The mixture was shaken at room temperature for 17 hours. Next, 1 ml of 20 mM triethyl ammonium-bicarbonate buffer (pH 7.0, hereafter simply referred to as TEAB) to dilute. The dilution was purified by gel filtration  
 40 chromatography using a Sephadex G25-packed column (diameter of 1.6 cm, length of 5 cm, NAP-25, manufactured by Pharmacia Inc.). That is, after the reaction dilution described above was added, elution was performed using 20 mM TEAB buffer as the mobile phase and passed-through fractions were collected and evaporated to dryness. The residue was dissolved in 300  $\mu$ l of 0.1 M triethylamine-acetate buffer (pH 7.0, hereafter simply referred to as TEAA) containing 10% acetonitrile and the solution was purified through  
 45 a column packed with reverse phase silica gel Waters Co., Ltd., Prep PAK-500/C-18). That is, 0.1 M TEAA buffer having a linear gradient of 10% to 40% acetonitrile was used as the mobile phase and quantitative determination was performed at absorbancy at a wavelength of 254 nm to give the fraction of Compound XXIII having a dimethoxytrityl group at the 5'-terminal. After the solvent was distilled off, the residue was co-evaporated together with water 3 times and 3 ml of 80% acetic acid was added thereto followed by stirring  
 50 for 10 minutes. After the reaction liquid was evaporated to dryness, the residue was further co-evaporated under reduced pressure together with water to remove acetic acid. To the residue was added 1.5 ml of 20 mM TEAB buffer. The mixture was washed twice with 1.5 ml of ethyl acetate. The aqueous layer was evaporated to dryness and 200  $\mu$ l of 0.1 M TEAA buffer containing 10% acetonitrile was added to the resulting residue. The mixture was purified by high performance liquid chromatography. That is, YMC Pack  
 55 ODS (manufactured by Yamamura Science Co., Ltd.; diameter of 6 mm, length of 30 cm) column was used and 0.1 M TEAA having a linear gradient of 10% to 50% acetonitrile was used as the mobile phase. The main peak eluted at a flow rate of 1.2 ml/min was fractionated to give 126 OD<sup>260 nm</sup> units (about 514) nmols) of Compound XXIII in yield of 12.9%.

In a manner similar to the procedure described above, Compound XXIV was obtained in 65 OD<sup>260</sup> nm units (about 264 nmols) showing the overall yield of about 11%, using as the starting material 2.4  $\mu$ moles of 5'-O-dimethoxytrityl-2'-O-(tert-butyldimethylsilyl)inosine-bound CPG and repeating 19 cycles in the condensation cycle using 5'-O-dimethoxytrityl-3'-O-(tert-butyldimethylsilyl)inosine-2'-O-(H-phosphonate) (Compound XXII) together with pivaloyl chloride.

Compounds XXIII and XXIV were dissolved in D<sub>2</sub>O containing 10 mM TEAB buffer (pH 7.5) and <sup>31</sup>P-NMR was measured (device for measurement: JEOL GX-400FT-NMR manufactured by JEOL Inc.). When trimethyl phosphate was used as the external standard, Compound XXIV showed the characteristic signal alone at 53-54 ppm and Compound XXIV showed the characteristic signal alone at 52-55 ppm, to multiplet phosphorothioate.

Furthermore, Compounds XXV, XXVI, XXXIII, XXXIV and XXXV were produced in a manner similar to the procedure described above.

On the other hand, Compound XXX was produced by, in the production steps of Compound XXV, performing the acid treatment after completion of the final condensation cycle, condensing with monomethoxytrityl-hexanediol-H-phosphonate, then performing the acid treatment, finally condensing with cholesteryl-H-phosphonate and thereafter carrying out in a similar manner. Compound XXXI was also produced by finally condensing with stearyl-H-phosphonate and thereafter carrying out in a similar manner.

With respect to Compound XXXII, the compound was produced by treating Compound XXV with acetic anhydride and pyridine. Compound XXXVI was produced using monomethoxytrityl-hexanediol-bound controlled pore glass as the starting material, by treating with an acid after completion of the condensation cycle as in the production of Compound XXV, then finally condensing with monomethoxytrityl-hexanediol-H-phosphonate and thereafter carrying out in a manner similar to the production of Compound XXV.

#### Example 7 Test on anti-AIDS virus activity

Compound XIII which was a deoxyoligonucleotide (phosphate type) having base sequence complementary to the same regions as in Compound VI, Compound XI and Compound VI, Compound XIV which was a deoxyoligonucleotide (phosphate type) having base sequence complementary to the same region as in Compound XI and dCS (15-mer) which was deoxyoligocytidylate phosphorothioate, were dissolved in 10% fetal calf serum-containing RPMI 1640 medium in a definite concentration. MT-4 cells infected with AIDS virus (HTLV-III<sub>B</sub> strain, 3 x 10<sup>5</sup> PFU/ml) at a MOI of 0.002 were mixed with the aforesaid medium containing oligonucleotide in 3 x 10<sup>5</sup>/ml at the initial stage followed by culturing at 37 °C. After the cell state was observed on Day 3, the system was again adjusted to cell counts of 3 x 10<sup>5</sup> to 5 x 10<sup>5</sup>/ml. Oligonucleotide was also freshly supplemented to have the same concentration as in the initial stage. Cytopathic effect with viral infection was evaluated 6 days after the infection. The results are shown in Table 2, indicating that symbol + denotes the case in which viral infection and proliferation are inhibited and the cells grow normally and symbol - denotes the case in that the cells are destructed due to viral infection. In any case, no cytotoxicity was noted in tested concentrations.

Table 2

| Compound               | Concentration |    |    |    |   |
|------------------------|---------------|----|----|----|---|
|                        | 30            | 20 | 15 | 10 | 5 |
| Compound VI            |               | +  |    | +  | - |
| Compound XIII (note 1) |               | -  |    | -  |   |
| Compound XI            | +             |    | -  |    |   |
| Compound XIV (note 2)  |               | -  |    | -  |   |
| dCS (15-mer) (note 3)  |               |    |    | -  | - |

(Note 1) Compound XIII: deoxyoligonucleotide (phosphate type) complementary to the same region as in Compound VI.

(Note 2) Compound XIV: deoxyoligonucleotide (phosphate type) complementary to the same region as in Compound XI.

(Note 3) dCS (15-mer): deoxyoligocytidylate phosphorothioate (15-mer).

With respect to Compound VI, further detailed test was carried out. That is, Compound VI was dissolved

in 10% fetal calf serum-containing RPMI 1640 medium in concentrations of 100  $\mu$ M, 50  $\mu$ M, 25  $\mu$ M, 12.5  $\mu$ M, 6  $\mu$ M and 3  $\mu$ M, at the time when the test was initiated. MT-4 cells infected with AIDS virus (HTLV-III<sub>B</sub> strain) at a MOI of 0.002 were mixed with the medium containing Compound VI in  $30 \times 10^4$  cells/ml at the onset, followed by culturing in a CO<sub>2</sub> incubator at 37° C. For control, non- infected MT-4 cells were cultured in the medium containing Compound VI at the same time. A part of the culture was taken out on Day 3; viable cells were counted by trypan blue dye staining and a rate of viral antigen positive cells were determined by the indirect immunofluorescence method. On the other hand, in order to prevent influence on cell growth due to overgrowth, the culture cells were diluted again to  $30$  to  $50 \times 10^4$  cells/ml with the culture containing Compound VI in the same concentration and culture was further continued at 37° C. On Day 6 after the onset of test, the viable cell count and the rate of viral antigen positive cells were again determined. The results are shown in Table 3. The viable cell counts on Day 3 and Day 6 are shown in Figs. 1 and 2, respectively. The rate of viral antigen positive cells is shown in Fig. 3.

Table 3

| Anti-AIDS Viral Activity of Compound VI (viable cell count and rate of viral antigen positive cells) |              |     |     |     |      |     |     |     |
|--|--------------|-----|-----|-----|------|-----|-----|-----|
| Concentration of Compound VI ( $\mu$ M)  |              | 100 | 50  | 25  | 12.5 | 6   | 3   | 0   |
| Viable cell count on Day 3 ( $\times 10^4$ cells/ml)   | Non-infected | 152 | 170 | 173 | 165  | 171 | 174 | 172 |
|  | Infected     | 153 | 175 | 171 | 166  | 178 | 147 | 79  |
| Viable cell count on Day 6 ( $\times 10^4$ cells/ml)   | Non-infected | 207 | 249 | 239 | 228  | 236 | 245 | 252 |
|  | Infected     | 207 | 239 | 246 | 211  | 146 | 137 | 2   |
| Rate of viral antigen positive cells (%)   | on Day 3     | 0   | 1   | 1.8 | 2.3  | 2.2 | 4   | 75  |
|  | on Day 6     | 0   | 1   | 1   | 2.8  | 72  | 85  | 100 |

With respect to Compounds VI, VII, VIII, IX, X, XII and deoxyoligocytidylate phosphorothioate: dCS (15-mer), similar test was also carried out to determine the viable cell count and rate of viral antigen positive cells on Day 6 after infection.

These results are shown in Table 4 with respect to the cell count on Day 6 after infection and in Table 5 with respect to the rate of viral antigen positive cells on Day 6 after infection. These results are also shown by graphs in Figs. 4 and 5, respectively.

Table 4

| Anti-AIDS Viral Activity of Compounds VI, VII, VIII, IX, X, XII and dCS (15-mer) (viable cell count on Day 6 after infection: $\times 10^4$ cells/ml) |     |     |     |     |     |     |     |     |     |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Concentration of Compound ( $\mu$ M)  | 240 | 120 | 60  | 30  | 15  | 7.5 | 3.8 | 1.9 | 1   |
| Compound VI   |     | 225 | 201 | 199 | 171 | 145 | 110 | 31  | 24  |
| Compound VII  |     | 232 | 204 | 205 | 162 | 122 | 33  | 22  | 26  |
| Compound VIII   |     | 213 | 204 | 206 | 179 | 176 | 152 | 151 | 134 |
| Compound IX   | 173 | 129 | 19  | 16  | 2   | 4   | 0   | 0   |     |
| Compound X  |     | 205 | 226 | 196 | 157 | 124 | 96  | 20  | 15  |
| Compound XII  |     | 239 | 254 | 257 | 130 | 8   | 2   | 0   | 0   |
| dCS (15-mer)  | 206 | 169 | 166 | 129 | 107 | 56  | 29  | 23  |     |
| Non-infected MT-4 cells: 220  |     |     |     |     |     |     |     |     |     |

Table 5

| Anti-AIDS Viral Activity of Compounds VI, VII, VIII, IX, X, XII and dCS (15-mer) (rate of viral antigen positive cells on Day 6 after infection: %) |     |     |     |     |     |     |     |     |     |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Concentration of Compound ( $\mu$ M)  | 240 | 120 | 60  | 30  | 15  | 7.5 | 3.8 | 1.9 | 1   |
| Compound VI   |     | 1   | 1   | 1   | 2   | 2   | 8   | 100 | 100 |
| Compound VII  |     | 2   | 2   | 2   | 4   | 8   | 60  | 100 | 100 |
| Compound VIII   |     | 1   | 1   | 1   | 1   | 2   | 2   | 2   | 8   |
| Compound IX   | 4   | 80  | 100 | 100 | 100 | 100 | 100 | 100 |     |
| Compound X  |     | 1   | 1   | 2   | 2   | 4   | 20  | 100 | 100 |
| Compound XII  |     | 1   | 1   | 1   | 1   | 33  | 87  | 100 | 100 |
| dCS (15-mer)  | 2   | 4   | 8   | 10  | 40  | 100 | 100 | 100 |     |
| Non-infected MT-4 cells: 0  |     |     |     |     |     |     |     |     |     |

With respect to Compounds XV, XVI, XVII, XVIII, XXIII and XXIV, similar test was also carried out. Regarding the results of Compounds XV, XVI, XVII and XVIII, the viable cell count on Day 6 after infection is shown in Table 6 and the rate of viral antigen positive cells on Day 6 after infection is shown in Table 7. These results are also shown by graphs in Figs. 6 and 7, respectively. On the other hand, with respect to the results of Compounds XXIII and XXIV, the viable cell count on Day 6 after infection is shown in Table 8 and the rate of viral antigen positive cells on Day 6 after infection is shown in Table 9. The viable cell counts of Compound XXIII and Compound XXIV are shown by graphs in Figs. 8 and 9, respectively. Furthermore, the rates of viral antigen positive cells of Compounds XXIII and XXIV on Day 6 after infection are shown by graphs in Fig. 10.

Table 6

| Anti-AIDS Viral Activity of Compounds XV, XVI, XVII and XVIII (viable cell count on Day 6 after infection: $\times 10^4$ cells/ml) |     |     |     |     |     |     |     |     |
|--|-----|-----|-----|-----|-----|-----|-----|-----|
| Concentration of Compound ( $\mu$ M)   | 120 | 60  | 30  | 15  | 7.5 | 3.8 | 1.9 | 1   |
| Compound XV  |     | 215 | 240 | 285 | 277 | 135 | 21  | 3   |
| Compound XVI   |     | 198 | 242 | 171 | 192 | 181 | 148 | 137 |
| Compound XVII  | 201 | 195 | 134 | 125 | 85  | 43  | 20  |     |
| Compound XVIII   | 151 | 89  | 16  | 13  | 3   | 1   | 2   |     |
| Non-infected MT-4 cells: 220   |     |     |     |     |     |     |     |     |

Table 7

| Anti-AIDS Viral Activity of Compounds XV, XVI, XVII and XVIII (rate of viral antigen positive cells on Day 6 after infection: %) |     |    |     |     |     |     |     |    |
|--|-----|----|-----|-----|-----|-----|-----|----|
| Concentration of Compound ( $\mu$ M)   | 120 | 60 | 30  | 15  | 7.5 | 3.8 | 1.9 | 1  |
| Compound XV  |     | 1  | 1   | 1   | 2   | 78  | 99  | 99 |
| Compound XVI   |     | 1  | 1   | 1   | 1   | 1   | 2   | 5  |
| Compound XVII  | 1   | 1  | 2   | 4   | 80  | 100 | 100 |    |
| Compound XVIII   | 1   | 80 | 100 | 100 | 100 | 100 | 100 |    |
| Non-infected MT-4 cells: 0   |     |    |     |     |     |     |     |    |

Table 8

| Anti-AIDS Viral Activity of Compounds XXIII and XXIV (viable cell count on Day 6 after infection: x 10 <sup>4</sup> cells/ml) |     |     |     |     |     |     |     |
|---|-----|-----|-----|-----|-----|-----|-----|
| Concentration of Compound ( $\mu$ M)  | 60  | 30  | 15  | 7.5 | 3.8 | 1.9 | 0   |
| Compound XXIII:   |     |     |     |     |     |     |     |
| Non-infected  | 147 | 141 | 161 | 152 | 138 | 133 | 161 |
| Infected  | 159 | 159 | 156 | 142 | 127 | 10  | 2   |
| Compound XXIV:  |     |     |     |     |     |     |     |
| Non-infected  | 149 | 160 | 142 | 155 | 159 | 143 | 161 |
| Infected  | 149 | 173 | 157 | 153 | 11  | 3   | 2   |

Table 9

| Anti-AIDS Viral Activity of Compounds XXIII and XXIV (rate of viral antigen positive cells on Day 6 after infection: %) |     |     |     |     |     |     |     |
|---|-----|-----|-----|-----|-----|-----|-----|
| Concentration of Compound ( $\mu$ M)  | 60  | 30  | 15  | 7.5 | 3.8 | 1.9 | 0   |
| Compound XXIII  | 0.2 | 0.2 | 0.2 | 0.2 | 41  | >90 | >90 |
| Compound XXIV   | 0.2 | 0.2 | 4.3 | >90 | >90 | >90 | >90 |

As described above, the novel 2'-O-methylribooligonucleotide phosphorothioate derivatives and the novel ribooligonucleotide phosphorothioate derivatives according to the present invention have anti-AIDS viral activity and are expected to be used as drugs such as drugs for treatment of AIDS. Therefore, the present invention is extremely useful in the drug industries.

Reference is made above to the accompanying drawings in which:

Figs. 1 and 2 show the measurement results on the viable cell count of Compound VI on Day 3 and Day 6, respectively;

Fig. 3 shows the measurement results on the rate of viral antigen positive cells;

Figs. 4 and 5 show the measurement results on the viable cell count and the rate of viral antigen positive cells of Compounds VI, XII, VIII, IX, X, XII and deoxyoligocytidylate phosphorothioate: dCS (15-mer) on Day 6 after infection, respectively;

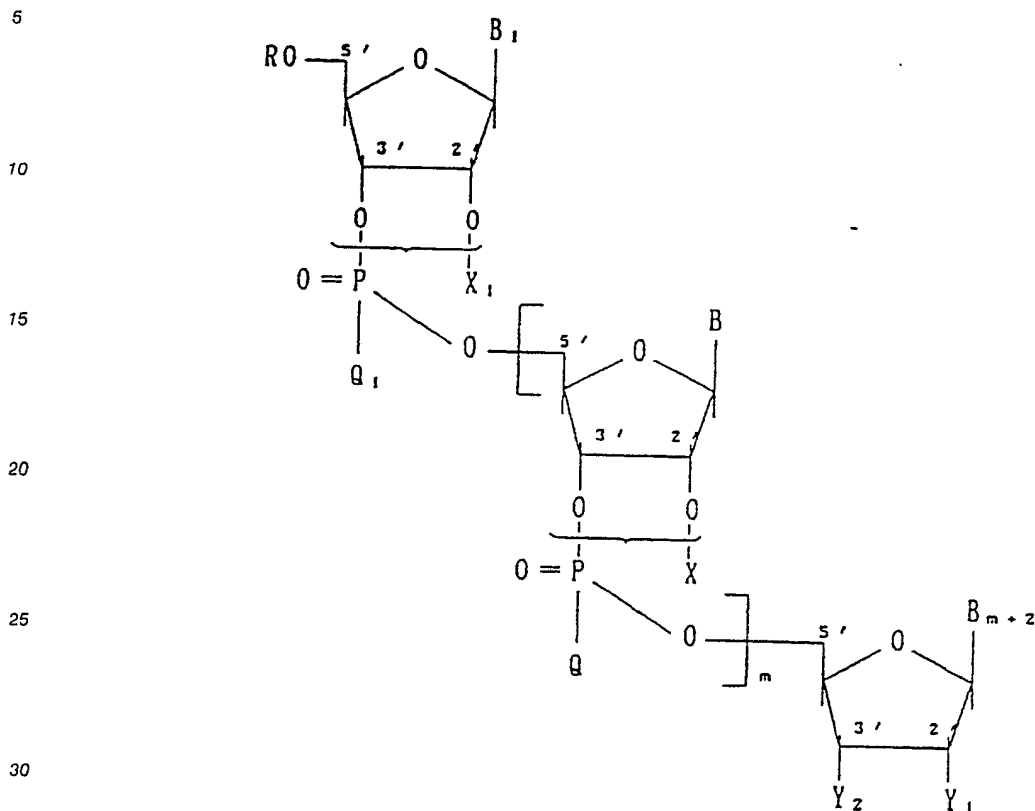
Figs. 6 and 7 show the measurement results, of Example 7, on viable cell count and rate of viral antigen positive cells of Compounds XV, XVI, XVII and XVIII on Day 6 after infection, respectively;

Figs. 8 and 9 show the measurement results in Example 7 on viable cell count of Compounds XXIII and XXIV on Day 6 after infection, respectively; and

Fig. 10 shows the rate of viral antigen positive cells of Compounds XXIII and XXIV on Day 6 after infection, of Example 7.

## Claims

1. An oligoribonucleotide derivative represented by the general formula:



wherein:

m is an integer of 1 to 100 (provided that when X<sub>1</sub> and X are all substituted at the 2'-position and represent a hydrogen atom, m represents an integer of 4 to 50);

B represents B<sub>2</sub>, B<sub>3</sub>, ..., B<sub>(m+1)</sub>, and B<sub>1</sub> to B<sub>(m+2)</sub> which may be the same or different, each represents any one of hypoxanthine-9-yl, guanine-9-yl, adenine-9-yl, cytosine-1-yl, uracil-1-yl and thymine-1-yl (provided that when X<sub>1</sub> and all of X are substituted at the 3'-position and represent a hydrogen atom, all of them do not represent adenine-9-yl);

Q represents Q<sub>2</sub>, Q<sub>3</sub>, ..., Q<sub>(m+1)</sub>, and Q<sub>1</sub> to Q<sub>(m+1)</sub> independently represents any one of a thioanion, an oxoanion, an alkyl group, an aryloxy group, an alkylamino group, an arylamino group, an alkylthio group and an arylthio group (provided that at least one of Q<sub>1</sub> to Q<sub>(m+1)</sub> represents a thioanion);

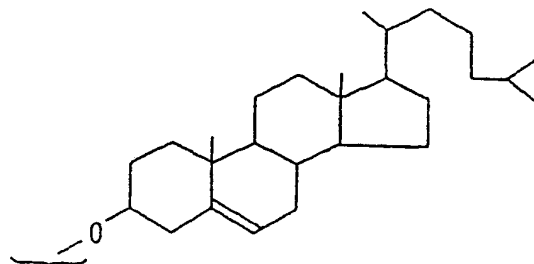
X represents X<sub>2</sub>, X<sub>3</sub>, ..., X<sub>(m+1)</sub>, and X<sub>1</sub> to X<sub>(m+1)</sub> which may be the same or different, each represents any one of a hydrogen atom, a methyl group or an acyl group having 1 to 20 carbon atoms;

R represents a hydrogen atom, an acyl group having 1 to 20 carbon atoms, a phosphoryl group which may optionally have a substituent, a thiophosphoryl group which may optionally have a substituent or an alkylaminophosphoryl group which may optionally have a substituent;

Y<sub>1</sub> and Y<sub>2</sub> independently represent a methoxy group, a carboxyl group having 1 to 20 carbon atoms, a hydrogen atom, a hydroxyl group, an alkyloxy group which may optionally have a substituent, a phosphoryloxy group which may optionally have a substituent, a thiophosphoryloxy group which may optionally have a substituent and an alkylaminophosphoryloxy group which may optionally have a substituent; and

X is capable of binding to an oxygen atom either at 2'-position or at the 3'-position in the sugar moiety of nucleoside monomer and, in this case, another is bound to a phosphorus atom (P).

2. A derivative as claimed in claim 1, wherein said substituent on R, Y<sub>1</sub> and Y<sub>2</sub>, which may be the same or different, each represents any one of a cyanoethyl group, a chlorophenyl group, a monophosphoryl group, a pyrophosphoryl group, a hydrocarbon residue having 1 to 20 carbon atoms, a cholesteryl group and a group having the formula J-(K-O-L)<sub>E</sub> wherein J represents:



a hydroxyl group or any one of the oligoribonucleotidyl groups defined for the structural formula of said derivative according to claim 1, from which any one of RO, Y<sub>1</sub> and Y<sub>2</sub> is removed;

K represents any one of a phosphoryl group, a thiophosphoryl group and an alkylaminophosphoryl group having 1 to 10 carbon atoms;

L represents a hydrocarbon residue having 1 to 20 carbon atoms; and

E represents an integer of 1 to 10.

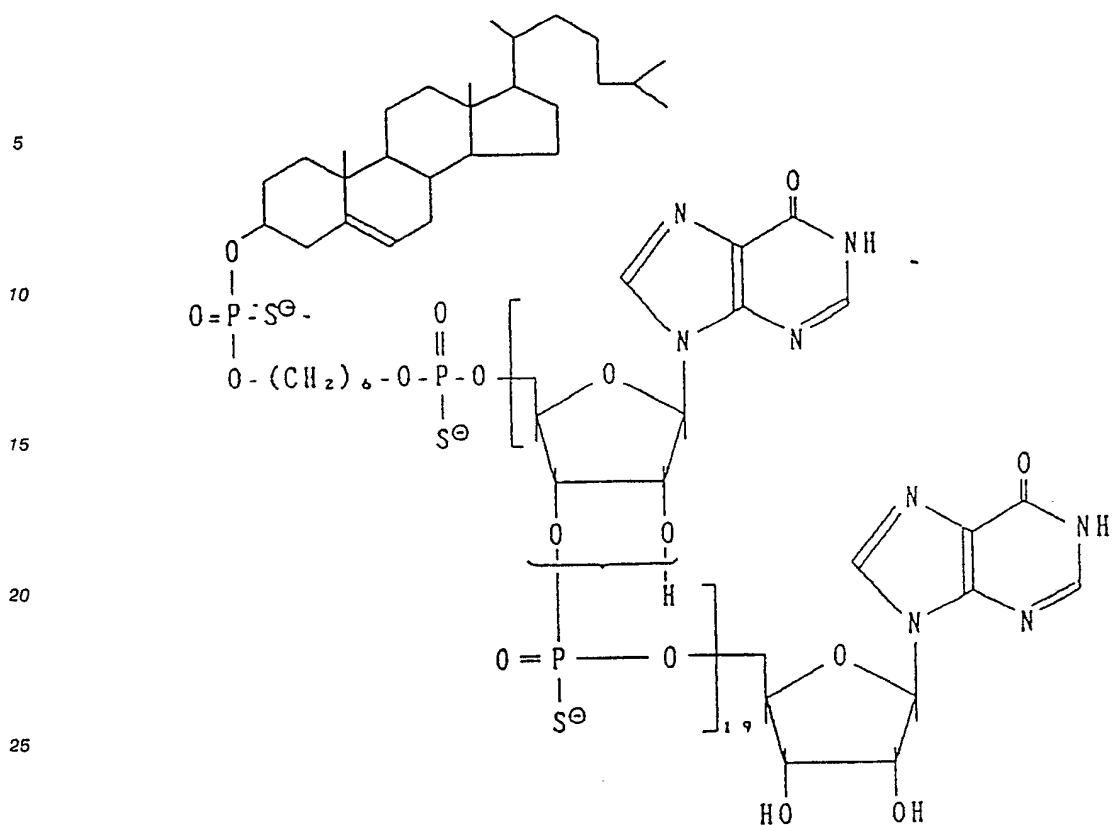
3. A derivative as claimed in claim 1, wherein m represents 25, B<sub>1</sub> to B<sub>27</sub> represents, in sequence, U, U, C, C, C, U, U, U, C, G, C, U, U, U, C, A, A, G, U, C, C, C, U, G, U, U, C and G; Q<sub>1</sub> to Q<sub>26</sub> each represent a thioanion; Y<sub>1</sub> and Y<sub>2</sub> represent OCH<sub>3</sub>; and Y<sub>3</sub> and R represents H.

4. A derivative as claimed in claim 1, wherein m represents 28, B<sub>1</sub> to B<sub>30</sub> represent, in sequence U, C, C, U, U, C, U, A, G, C, U, C, C, G, C, U, A, G, U, C, A, A, A, A, U, U, U and U; Q<sub>1</sub> to Q<sub>29</sub> each represent a thioanion; Y<sub>1</sub> and Y<sub>2</sub> represent OCH<sub>3</sub>; and Y<sub>3</sub> and R represents H.

5. A derivative as claimed in claim 1, wherein m represents 26, B<sub>1</sub> to B<sub>28</sub> represent, in sequence, U, U, U, U, A, A, U, U, U, A, U, A, U, U, U, U, U, U, C, U, U, U, C, C, C, C, C and U; Q<sub>1</sub> to Q<sub>27</sub> each represent a thioanion; Y<sub>1</sub> and Y<sub>2</sub> represent OCH<sub>3</sub>; and Y<sub>3</sub> and R represents H.

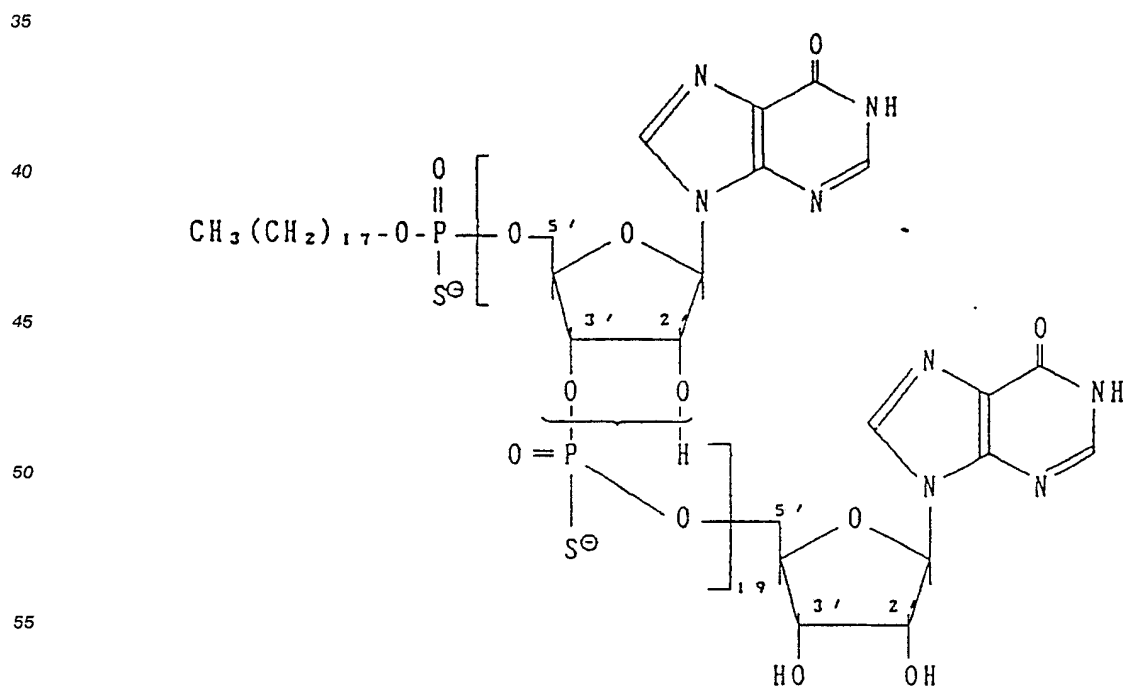
6. A derivative as claimed in claim 1, having the structural formula:





30 wherein a hydrogen atom (H) is capable of binding to the oxygen atom either at the 2'-position or at the 3'-position in the sugar moiety of the nucleoside monomer and, in this case, another is bound to a phosphorus atom (P).

7. A derivative as claimed in claim 1, having the structural formula:



wherein a hydrogen atom (H) is capable of binding to the oxygen atom either at the 2'-position or at the 3'-position in the sugar moiety of the nucleoside monomer and, in this case, another is bound to a phosphorus atom (P).

8. A derivative as claimed in claim 1, having the structural formula:

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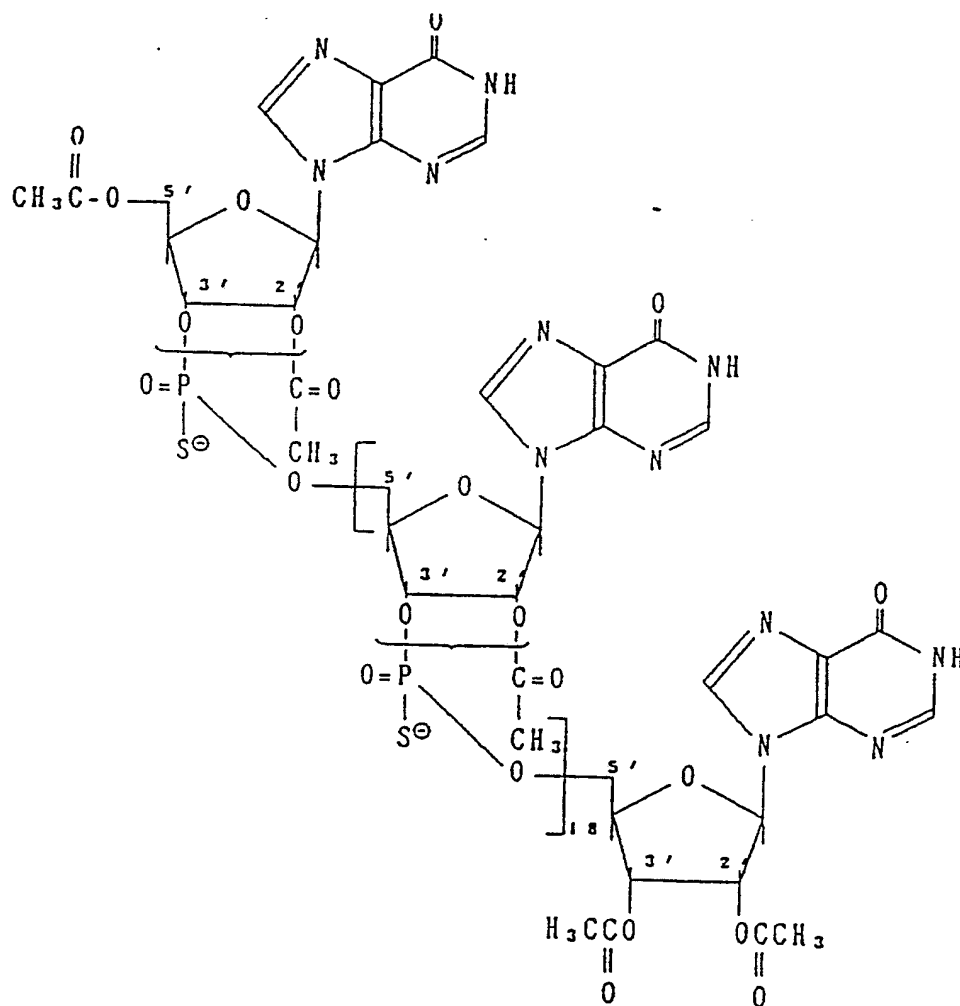
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wherein an acetyl group is capable of binding to the oxygen atom either at the 2'-position or at the 3'-position in the sugar moiety of the nucleoside monomer and, in this case, another is bound to a phosphorous atom (P).

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9. A derivative as claimed in claim 1, having the structural formula:

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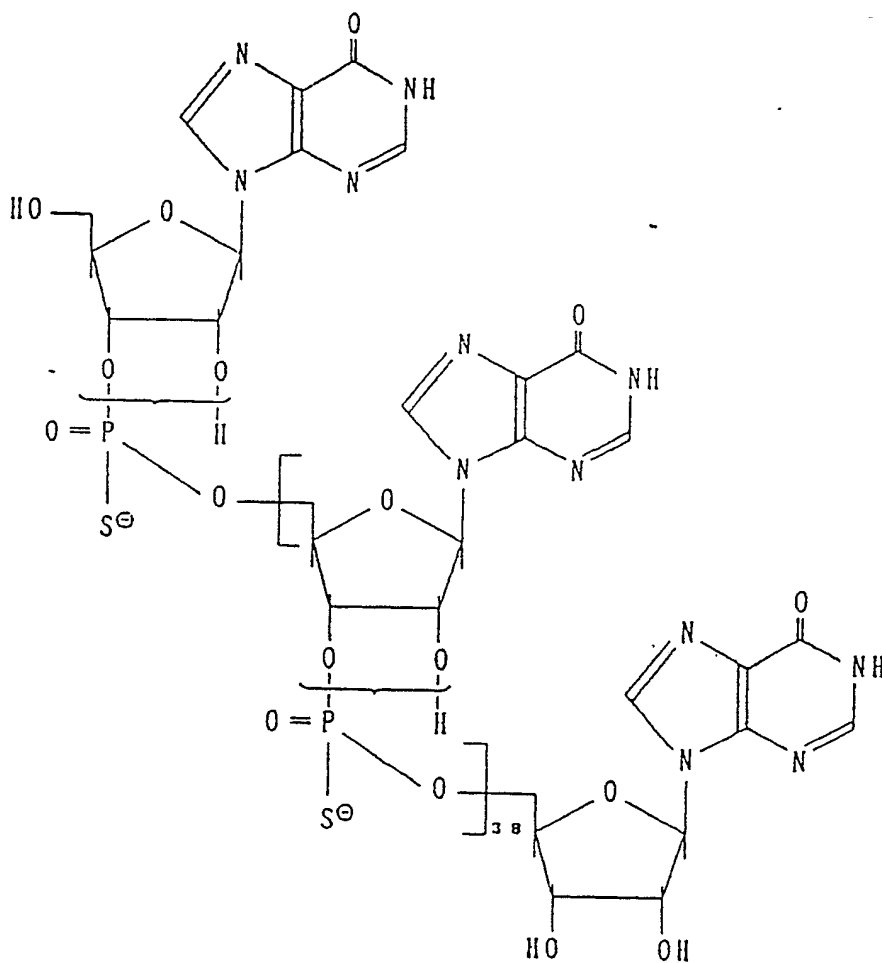
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35 wherein a hydrogen atom (H) is capable of binding to the oxygen atom either at the 2'-position or at the 3'-position in the sugar moiety of the nucleoside monomer and, in this case, another is bound to a phosphorus atom (P).

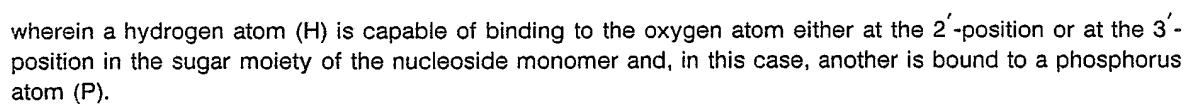
10. A derivative as claimed in claim 1, having the structural formula:

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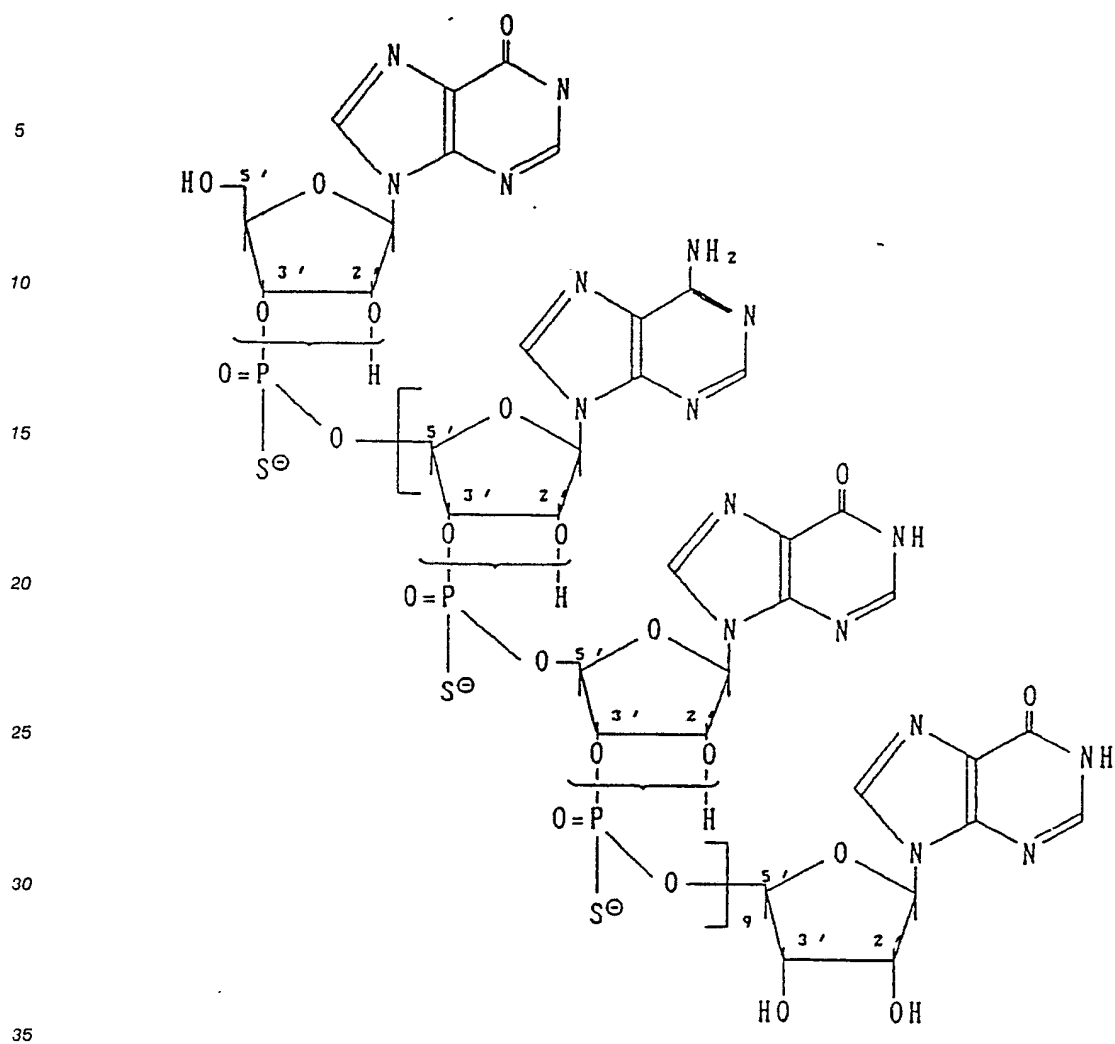
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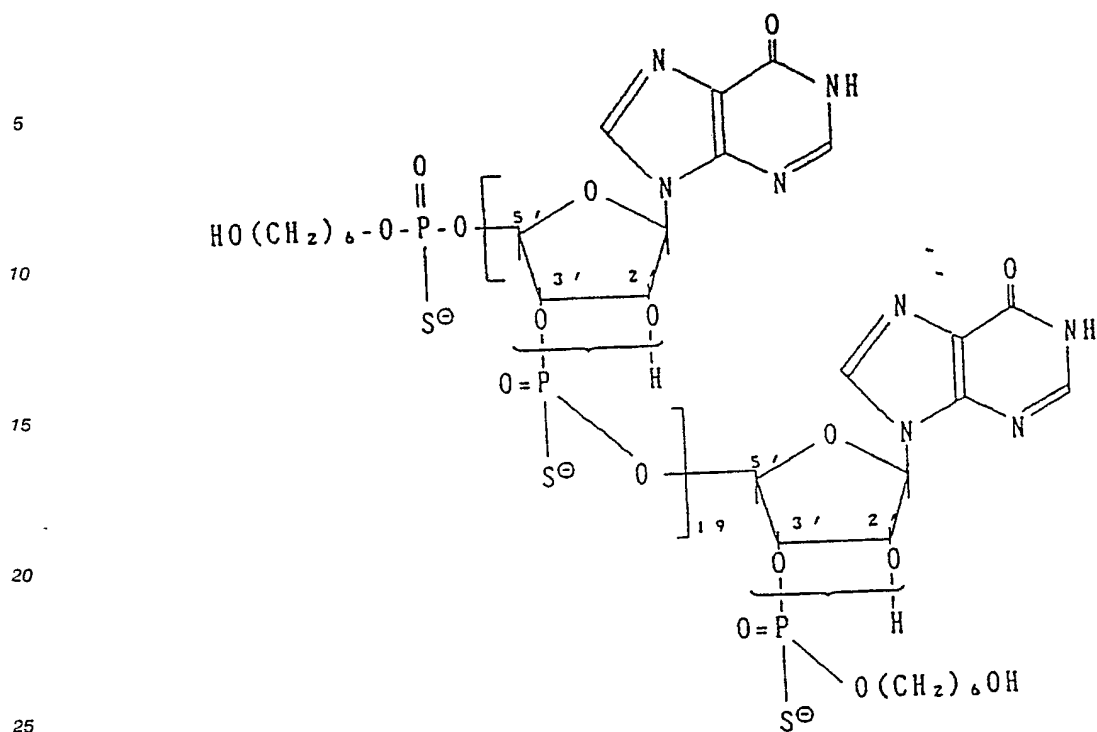


11. A derivative as claimed in claim 1, having the structural formula:



wherein a hydrogen atom (H) is capable of binding to the oxygen atom either at the 2'-position or at the 3'-position in the sugar moiety of the nucleoside monomer and, in this case, another is bound to a phosphorus atom (P).

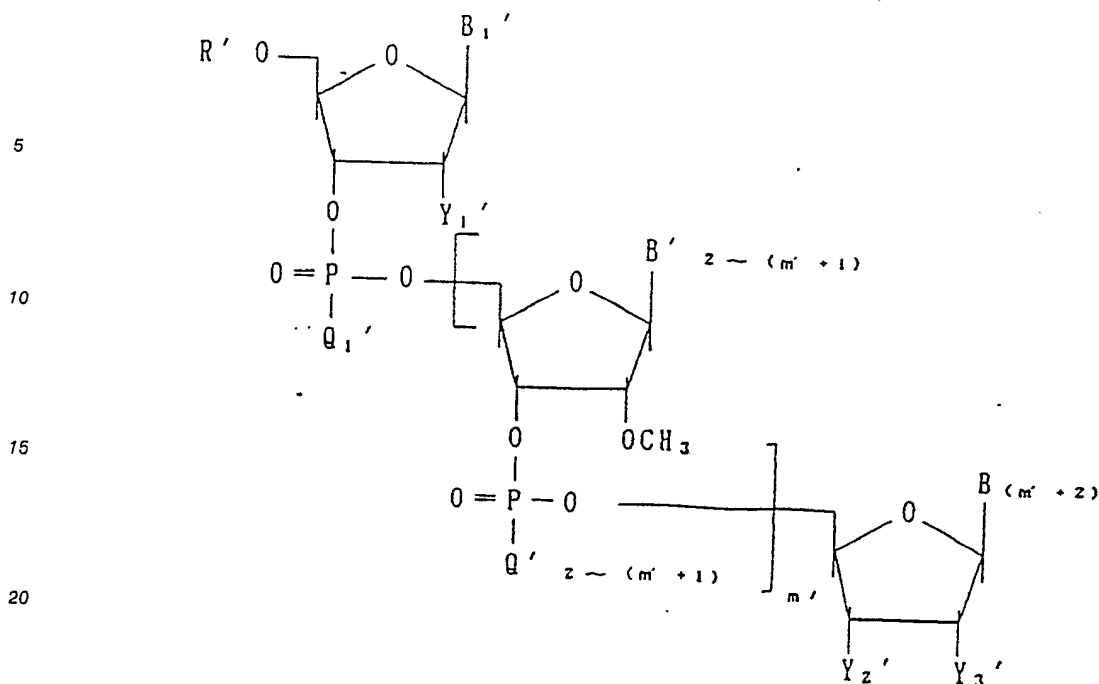
12. A derivative as claimed in claim 1, having the structural formula:



wherein a hydrogen atom (H) is capable of binding to the oxygen atom either at the 2'-position or at the 3'-position in the sugar moiety of the nucleoside monomer and, in this case, another is bound to a phosphorus atom (P).

13 An anti-AIDS viral agent comprising as an effective ingredient at least oligoribonucleotide derivative as claimed in any of claims 1 to 12.

14. A 2'-O-methylribooligonucleotide phosphorothiate derivative containing a sequence complementary to genomic RNA of AIDS virus or to DNA integrated into a chromosome and represented by the general formula:



wherein:

$m'$  is an integer of 1 to 50;

$B'_1$  to  $B'_{(m'+2)}$  which may be the same or different, each represents any one of adenine-9-yl, guanine-9-yl, cytosine-1-yl, uracil-1-yl, thymine-1-yl and hypoxanthine-9-yl;

$Q'_1$  represents any one of a thioanion, an oxoanion, an alkyl group, an alkyloxy group, an aryloxy group, an alkylamino group, an alkyloxy group, an alkylthio group and an arylthio group;

at least one of  $Q'_2$  to  $Q'_{(m'+1)}$ , represents a thioanion and the other(s) is(are) either a thioanion or an oxoanion;

$Y'_1$  to  $Y'_3$  independently represents a methoxy group, an alkyloxy group which may optionally have a substituent, a hydroxyl group, an alkylsilyl group or a hydrogen atom;

$R'$  represents a hydrogen atom, a phosphoryl group or a thiophosphoryl group which may optionally have a substituent.

15. A derivative as claimed in claim 14, wherein  $m'$  represents 18;  $B'_1$  to  $B'_{20}$  represent, in sequence, A, C, A, C, C, C, A, A, U, U, C, U, G, A, A, A, A, U, G and G;  $Q'_1$  to  $Q'_{19}$  represent a thioanion;  $Y'_1$  and  $Y'_2$  represent  $OCH_3$ ;  $Y'_3$  represents OH; and  $R'$  represents a hydrogen atom.

16. A derivative as claimed in claim 14, wherein  $m'$  represents 15;  $B'_1$  to  $B'_{17}$  represent, in sequence, C, C, C, A, A, U, U, C, U, G, A, A, A, A, U, G and G;  $Q'_1$  to  $Q'_{16}$  represent a thioanion;  $Y'_1$  and  $Y'_2$  represent  $OCH_3$ ;  $Y'_3$  represents OH; and  $R'$  represents a hydrogen atom.

17. A derivative as claimed in claim 14, wherein  $m'$  represents 23;  $B'_1$  to  $B'_{25}$  represent, in sequence, A, C, A, C, C, C, A, A, U, U, C, U, G, A, A, A, A, U, G, G, A, U, A, A and A;  $Q'_1$  to  $Q'_{24}$  represent a thioanion;  $Y'_1$  and  $Y'_2$  represent  $OCH_3$ ;  $Y'_3$  represents OH; and  $R'$  represents a hydrogen atom.

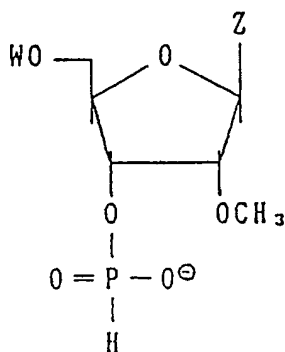
18. A derivative as claimed in claim 14, wherein  $m'$  represents 8;  $B'_1$  to  $B'_{10}$  represent, in sequence, C, A, A, U, U, C, U, G, A and A;  $Q'_1$  to  $Q'_9$  represent a thioanion;  $Y'_1$  and  $Y'_2$  represent  $OCH_3$ ;  $Y'_3$  represents OH; and  $R'$  represents a hydrogen atom.

19. A derivative as claimed in claim 14, wherein  $m'$  represents 19;  $B'_1$  to  $B'_{21}$  represent, in sequence, G, U, C, C, C, U, G, U, U, C, G, G, G, C, G, C, C, A, C, U and G;  $Q'_1$  to  $Q'_{20}$  represent a thioanion;  $Y'_1$  and  $Y'_2$  represent  $OCH_3$ ;  $Y'_3$  represents OH; and  $R'$  represents a hydrogen atom.

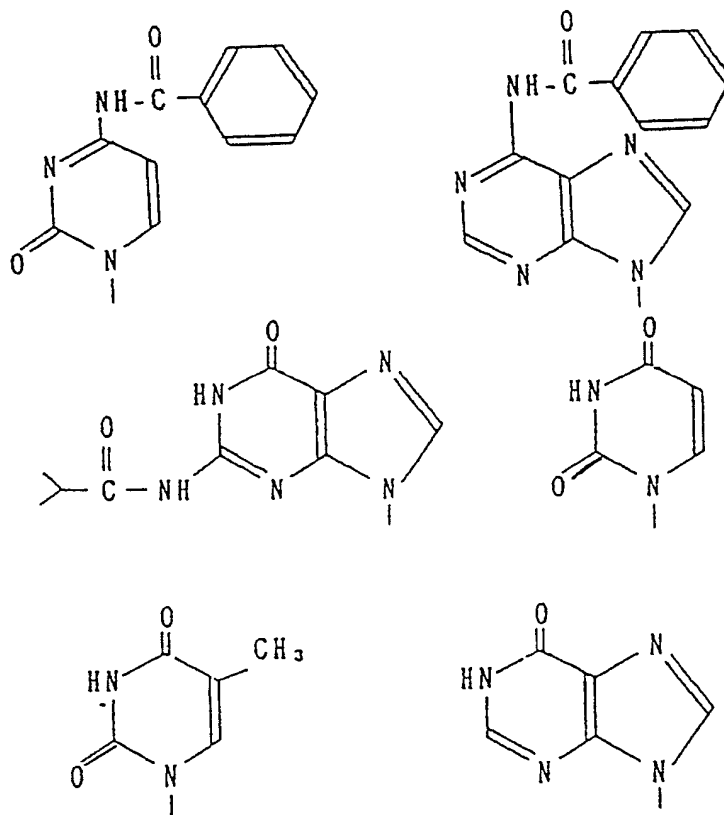
20. A derivative as claimed in claim 14, wherein  $m'$  represents 19;  $B'_1$  to  $B'_{21}$  represent, in sequence, G, A, G, C, U, C, C, C, A, G, G, C, U, C, A, G, A, U, C, U and G;  $Q'_1$  to  $Q'_{20}$  represent a thioanion;  $Y'_1$  and  $Y'_2$  represent  $OCH_3$ ;  $Y'_3$  represents OH; and  $R'$  represents a hydrogen atom.

21. A derivative as claimed in claim 14, wherein  $m'$  represents 18;  $B'_1$  to  $B'_{20}$  represent, in sequence, A, C, A, C, C, C, A, A, U, U, C, U, G, A, A, A, U, G, and G;  $Q'_1$  to  $Q'_5$  represent a thioanion;  $Q'_6$  to  $Q'_{14}$  represent an oxoanion; at least two of  $Q'_{15}$  to  $Q'_{19}$  represent a thioanion and the other three each represent an oxoanion;  $Y'_1$  and  $Y'_2$  represent  $OCH_3$ ;  $Y'_3$  represents OH; and  $R'$  represents a hydrogen atom.

22. A 2'-O-methylribonucleotide derivative having the general formula:

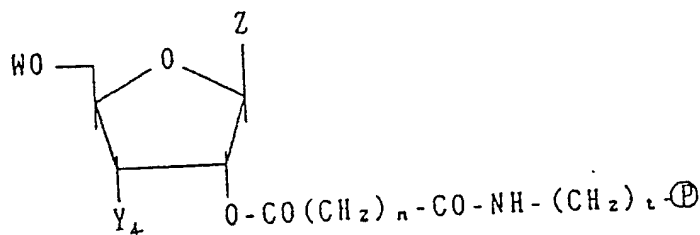


wherein W represents a monomethoxytrityl group or a dimethoxytrityl group; Z represents a substituent represented by any one of the general formulae:



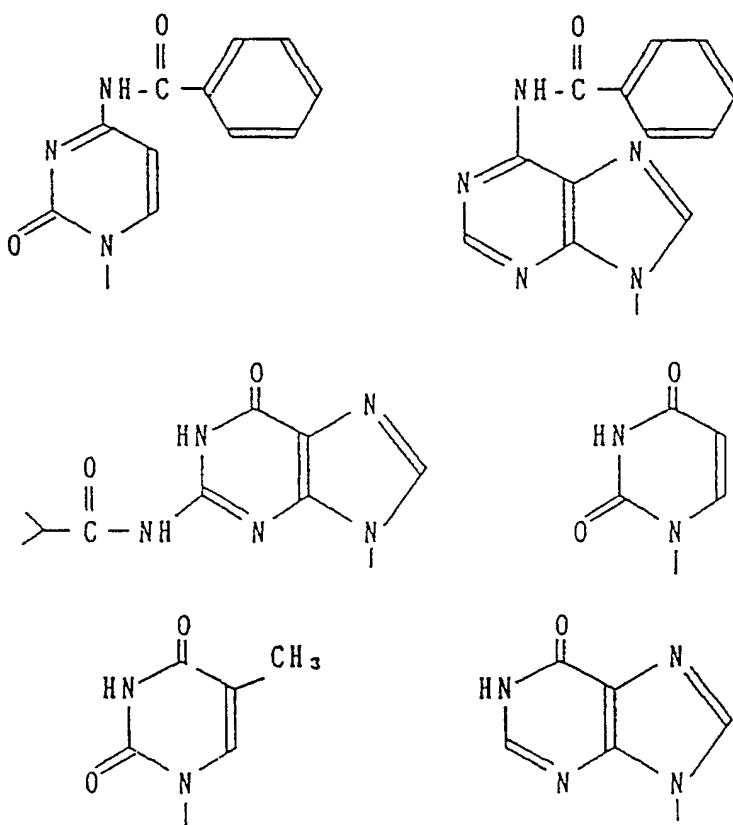
23. A nucleoside derivative represented by the general formula:





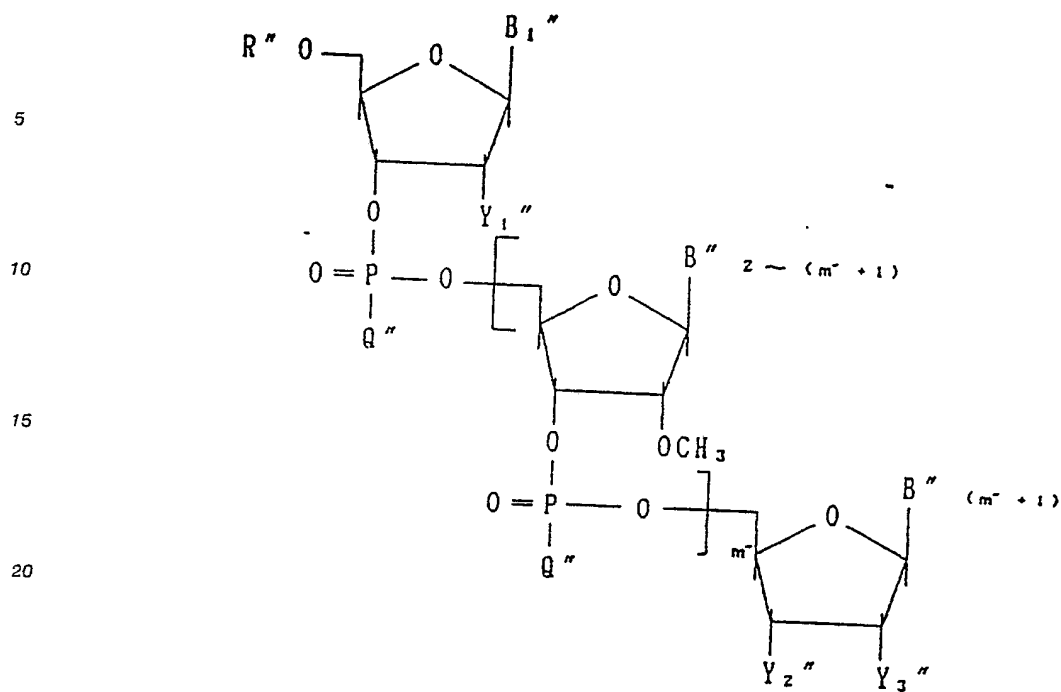
wherein W represents a monomethoxytrityl group or a dimethoxytrityl group; Y<sub>4</sub> represents a methoxy group, an alkyloxy group which may optionally have a substituent or a hydrogen atom; n and t, which may be the same or different, each represents an integer of 1 to 30;  $\textcircled{P}$  represents any one of a controlled pore glass derivative, a polystyrene derivative or a silica gel derivative; and Z represents a substituent represented by any one of the general formulae:

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24. A 2'-O-methylribooligonucleotide phosphorothioate derivative represented by the general formula:

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wherein:

$m''$  is an integer of 1 to 50;

$B''$  and  $B''_{(m''+2)}$ , which may be the same or different, each represents any one of adenine-9-yl, guanine-9-yl, cytosine-1-yl, uracil-1-yl, thymine-1-yl and hypoxanthine-9-yl;

$Q''$  contains at least one thioanion and represents any one of a thioanion, an oxoanion, an aryloxy group, an alkylamino group, an arylamino group, an alkylthio group and an arylthio group;

$Y''_1$  to  $Y''_3$  independently represents a methoxy group, an alkyloxy group which may optionally have a substituent, a hydroxyl group, an alkylsilyl group and a hydrogen atom;

$R''$  represents a hydrogen atom or a phosphoryl group which may optionally have a substituent.

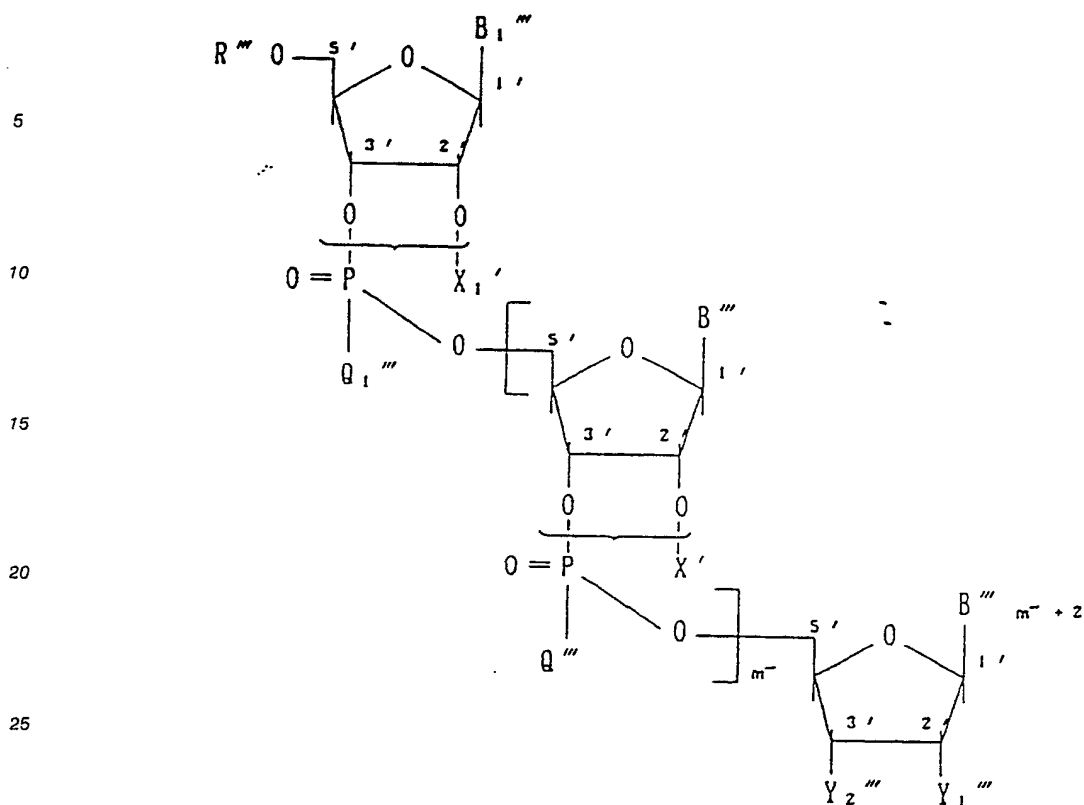
25. A derivative as claimed in claim 24, wherein  $m''$  represents 18;  $B''_1$  to  $B''_{20}$  represents adenine-9-yl;  $Q''$  represents a thioanion;  $Y''_1$  and  $Y''_2$  represent  $OCH_3$ ;  $Y''_3$  represents OH; and  $R''$  represents a hydrogen atom.

26. A derivative as claimed in claim 24, wherein  $m''$  represents 18;  $B''_1$  to  $B''_{20}$  represent hypoxanthine-9-yl;  $Q''$  represents a thioanion;  $Y''_1$  and  $Y''_2$  represent  $OCH_3$ ;  $Y''_3$  represents OH; and  $R''$  represents a hydrogen atom.

27. A derivative as claimed in claim 24, wherein  $m''$  represents 18;  $B''_1$  to  $B''_{20}$  represent uracil-1-yl;  $Q''$  represents a thioanion;  $Y''_1$  and  $Y''_2$  represent  $OCH_3$ ;  $Y''_3$  represents OH; and  $R''$  represents a hydrogen atom.

28. A derivative as claimed in claim 24, wherein  $m''$  represents 18;  $B''_1$  to  $B''_{20}$  represent cytosine-1-yl;  $Q''$  represents a thioanion;  $Y''_1$  and  $Y''_2$  represent  $OCH_3$ ;  $Y''_3$  represents OH; and  $R''$  represents a hydrogen atom.

29. An oligoribonucleotide derivative represented by the general formula:



30 wherein:

m''' is an integer of 1 to 100 (provided that when X' and X' are all substituted at the 2'-position and represent a hydrogen atom, m''' represents an integer of 4 to 50);

35 B<sup>m</sup> represents B<sup>m</sup>2, B<sup>m</sup>3, ..... B<sup>m</sup>(<sup>m</sup>+1), and B<sup>m</sup>1 to B<sup>m</sup>(<sup>m</sup>+2') which may be the same or different, each represents any one of hypoxanthine-9-yl, guanine-9-yl, adenine-9-yl, cytosine-1-yl, uracil-1-yl and thymine-1-yl (provided that all of them do not represent adenine-9-yl);

Q<sup>m''</sup> represents Q<sup>m''</sup><sub>2</sub>, Q<sup>m''</sup><sub>3</sub>, ..., Q<sup>m''</sup><sub>(m''+1)</sub>, and Q<sup>m''</sup><sub>1</sub> to Q<sup>m''</sup><sub>(m''+1)</sub>, independently represents any one of a thioanion, an oxoanion, an aryloxy group, an alkylamino group, an arylamino group, an alkylthio group and an arylthio group (provided that at least one of Q<sup>m''</sup><sub>1</sub> to Q<sup>m''</sup><sub>(m''+1)</sub>, represents a thioanion);

40  $X'_1$  represents  $X_2, X_3, \dots, X_{(m'''+1)}$ , and  $X'_1$  to  $X'_{(m'''+1)}$ , which may be the same or different, each represents a hydrogen atom or methyl group (provided that when all of  $X'_1$  to  $X'_{(m'''+1)}$  are substituted at the 2'-position, all of them do not represent a methyl group);

R<sup>iii</sup> represents any one of a hydrogen atom, an acyl group having 1 to 20 carbon atoms and a phosphoryl group which may optionally have a substituent;

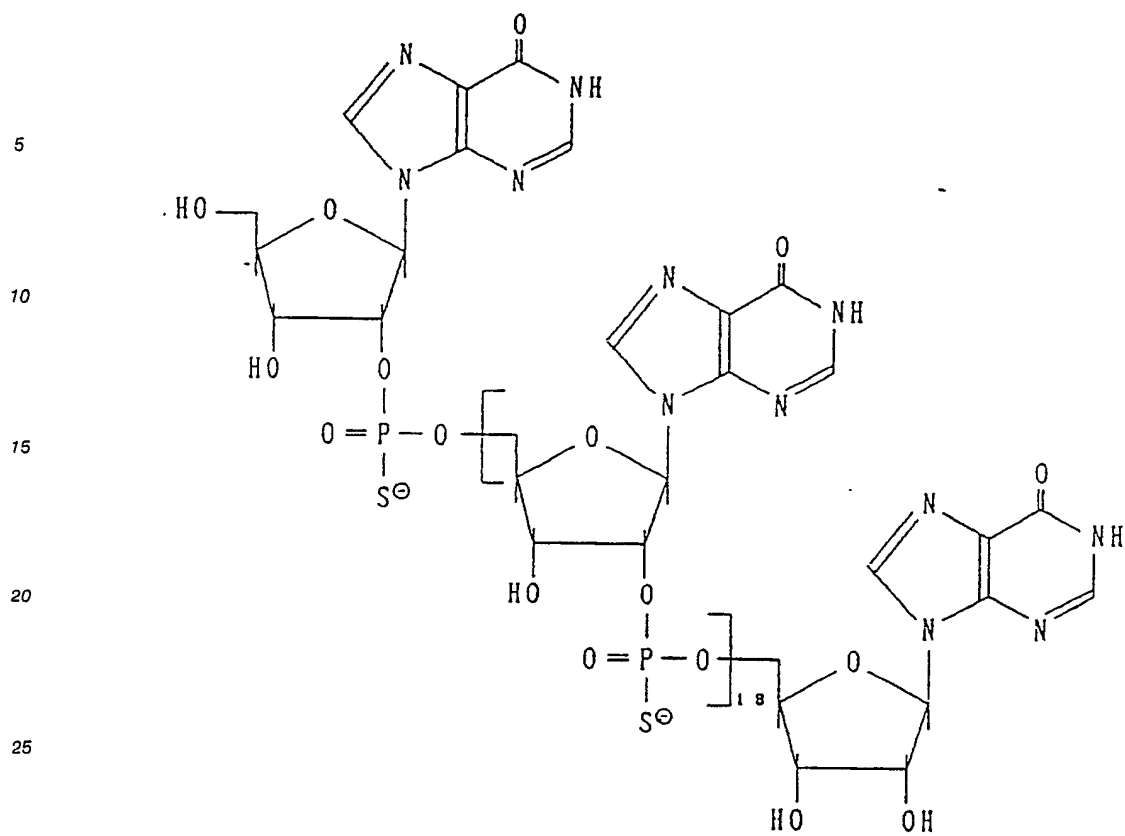
45  $Y''_1$  and  $Y''_2$  independently represents any one of a methoxy group, a carboxyl group having 1 to 20 carbon atoms, an alkyloxy group which may optionally have a substituent, a hydroxyl group, an alkylsilyl group, a hydrogen atom and a phosphoryl group which may optionally have a substituent; and

X is capable of binding to an oxygen atom either at the 2'-position or at the 3'-position in the sugar moiety of the nucleoside monomer and, in this case, another is bound to phosphorus atom (P).

30. A derivative as claimed in claim 29, wherein said substituent is any one of a cyanoethyl group, a  
50 chlorophenyl group and a hydrocarbon residue having 1 to 20 carbon atoms.

31 A derivative as claimed in claim 29, having structural formula:





30 33. A derivative as claimed in claim 29, having the structural formula:

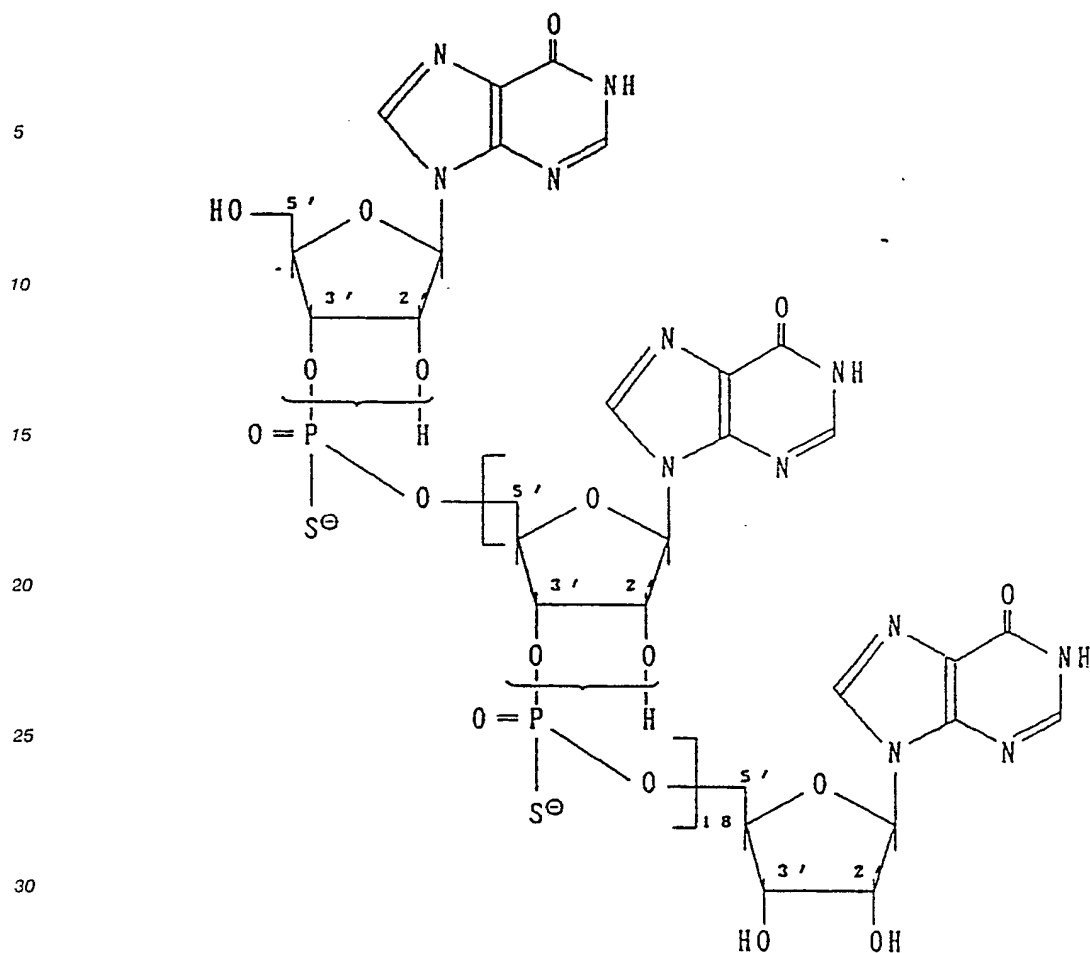
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35 wherein a hydrogen atom (H) is capable of binding to an oxygen atom either at the 2'-position or at the 3'-position in the sugar moiety of the nucleoside monomer and, in this case, another is bound to phosphorus atom (P).

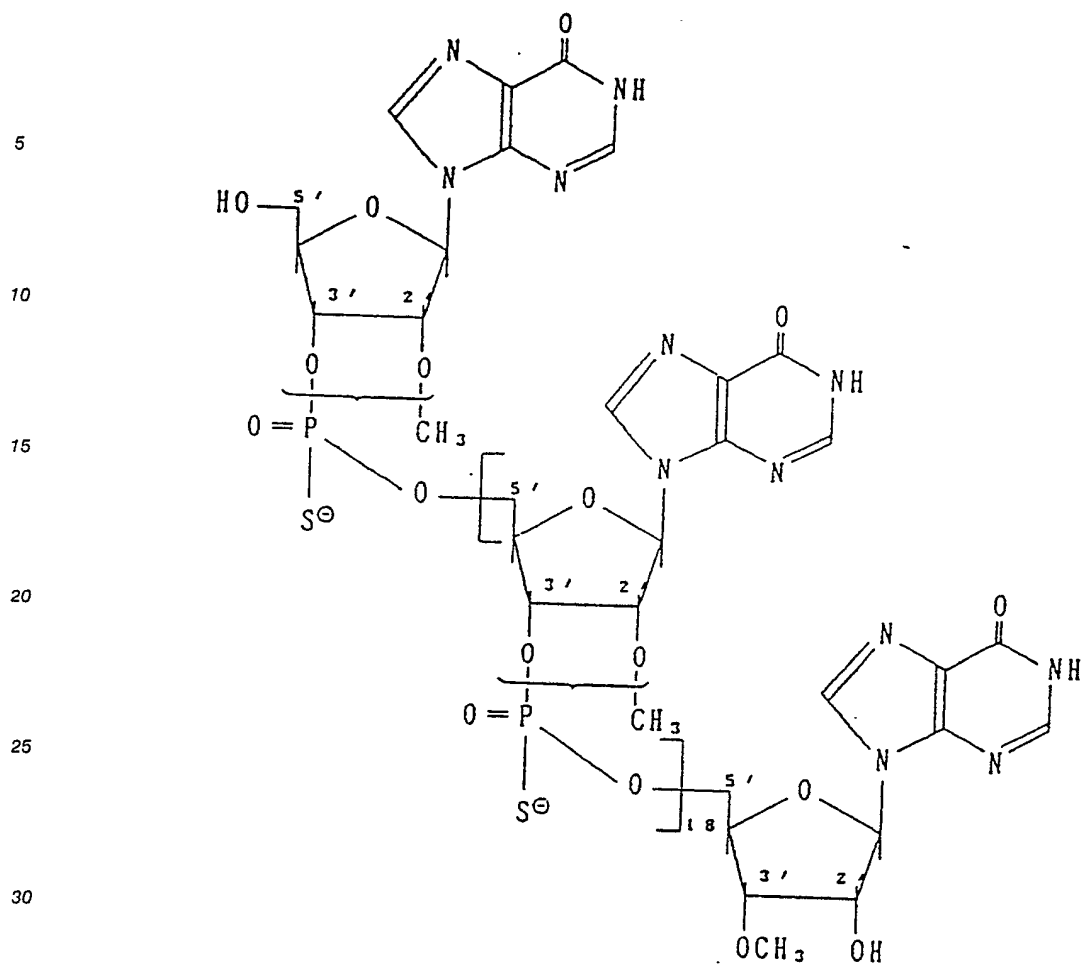
34. A derivative as claimed in claim 29, having the structural formula:

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wherein a methyl group ( $\text{CH}_3$ ) is capable of binding to an oxygen atom either at the 2'-position or at the 3'-position in the sugar moiety of the nucleoside monomer and, in this case, another is bound to phosphorus atom (P).

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Fig. 1.

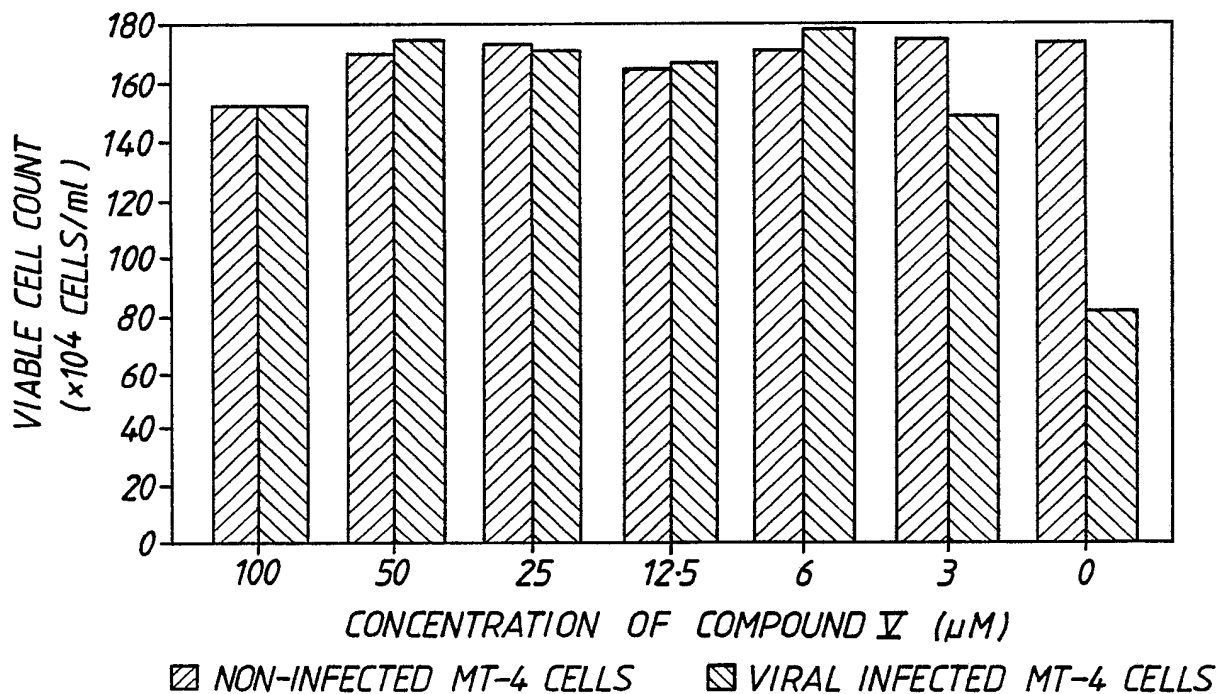
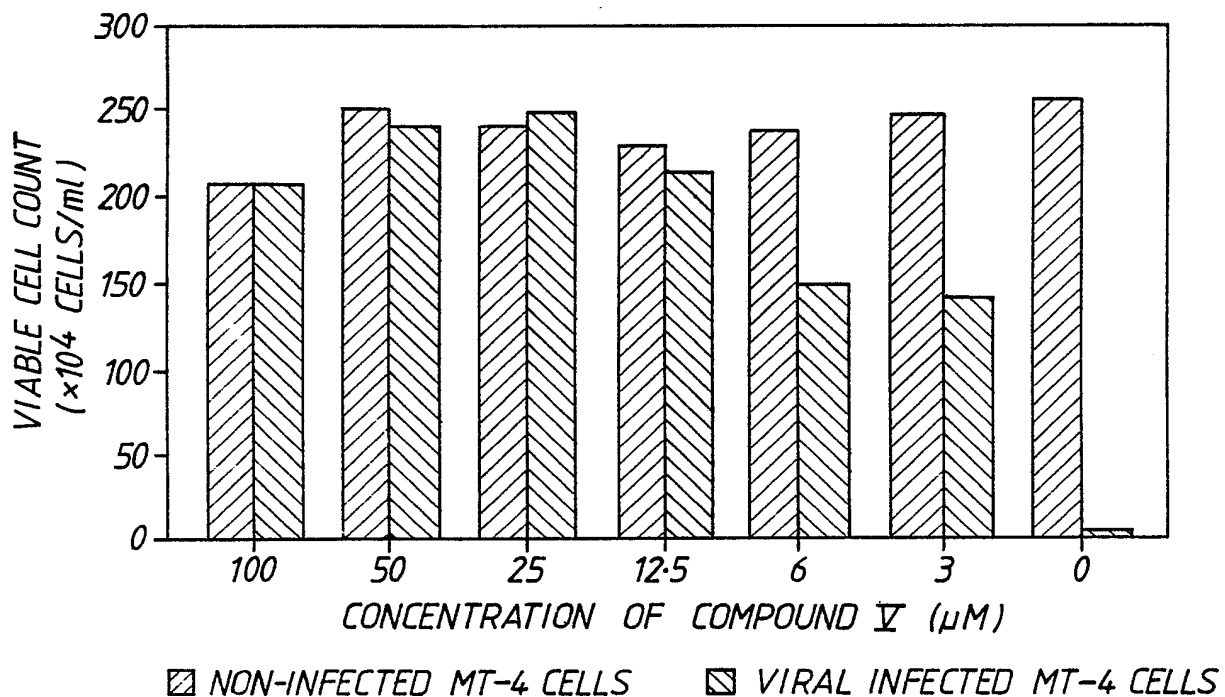


Fig. 2.





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Fig. 3.

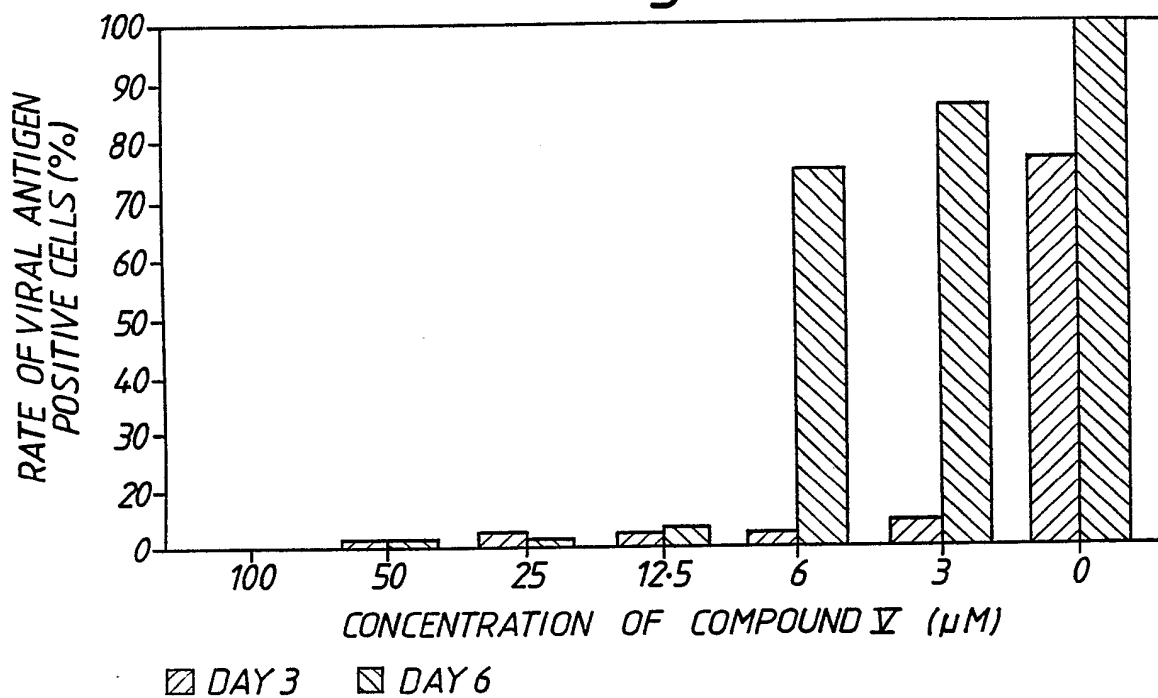


Fig. 4.

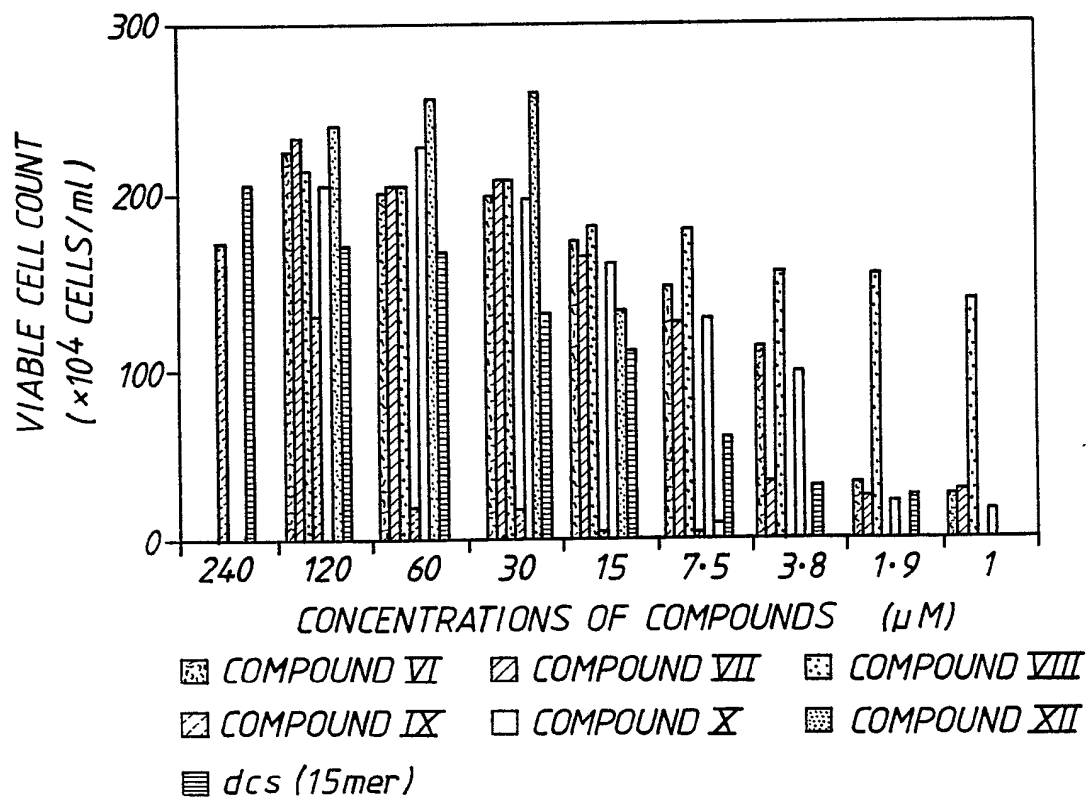


Fig. 5.

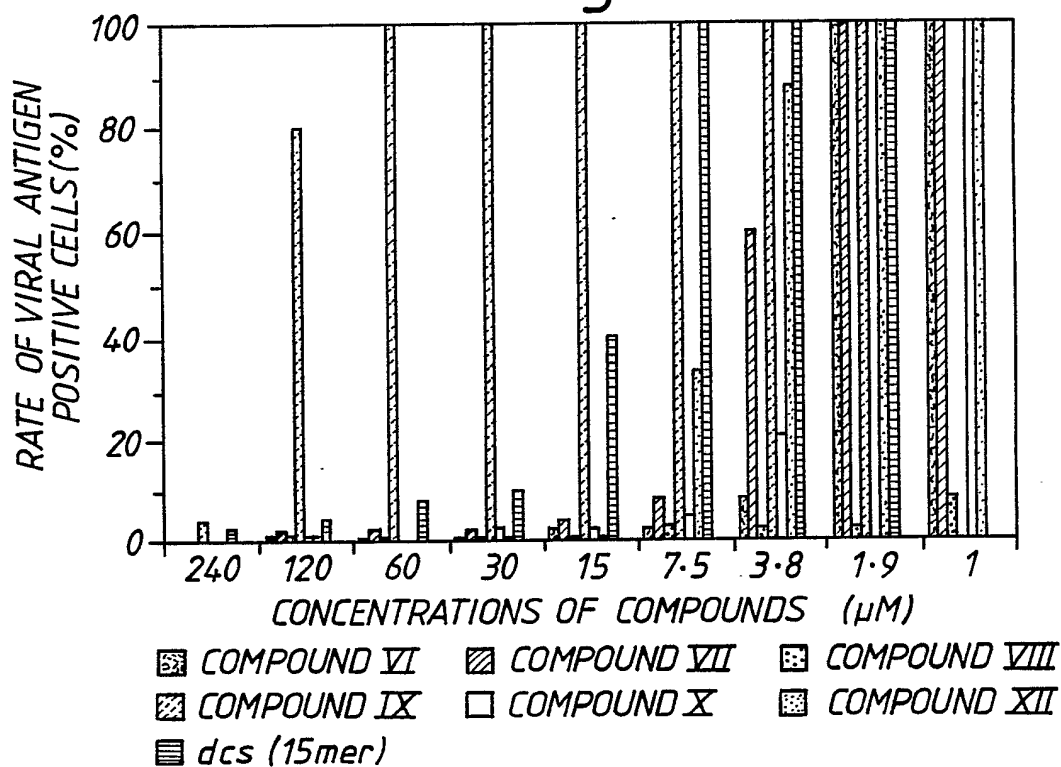


Fig. 6.

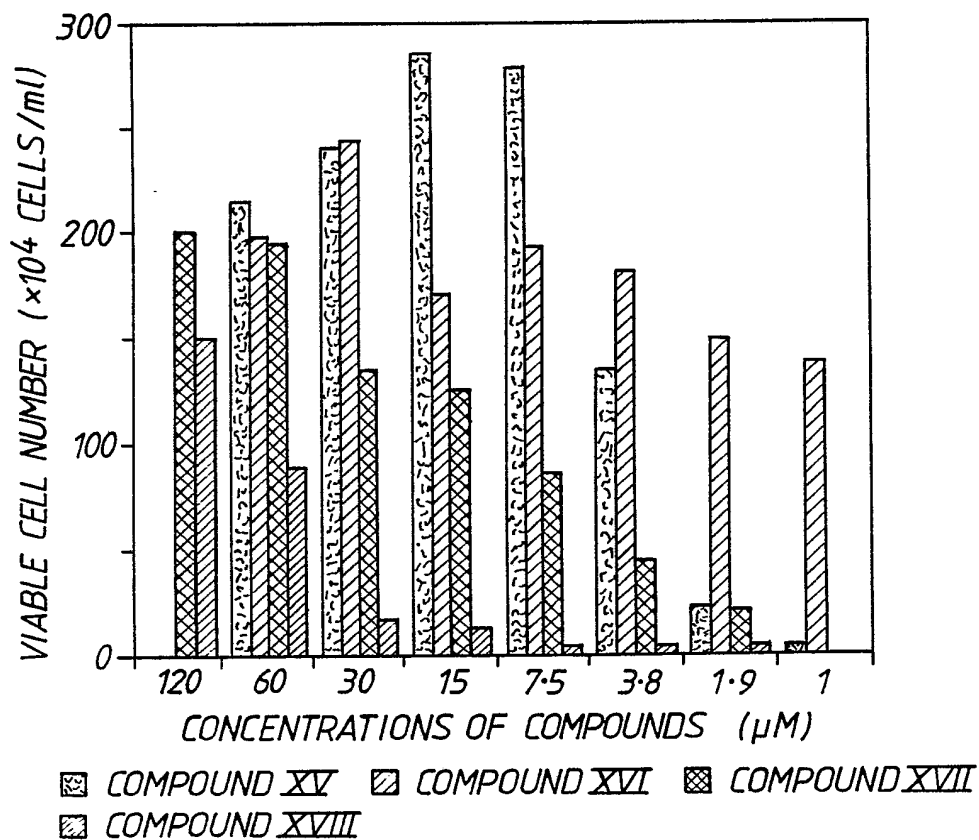


Fig. 7.

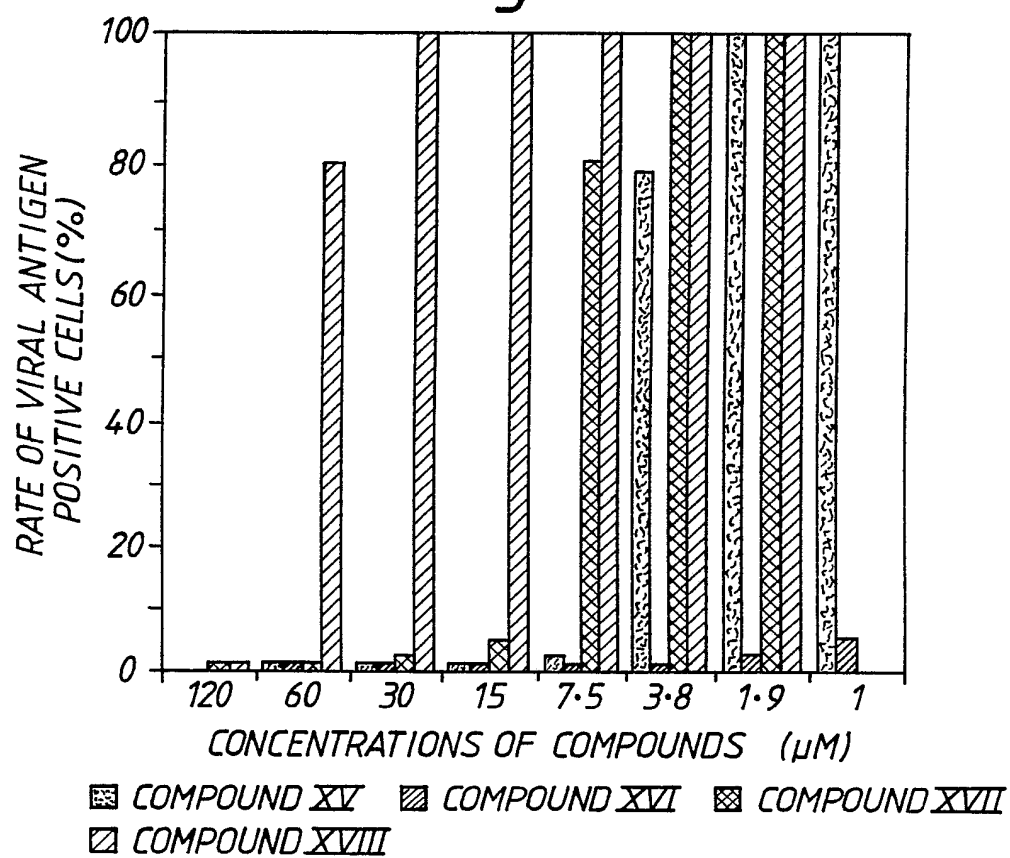


Fig. 8.

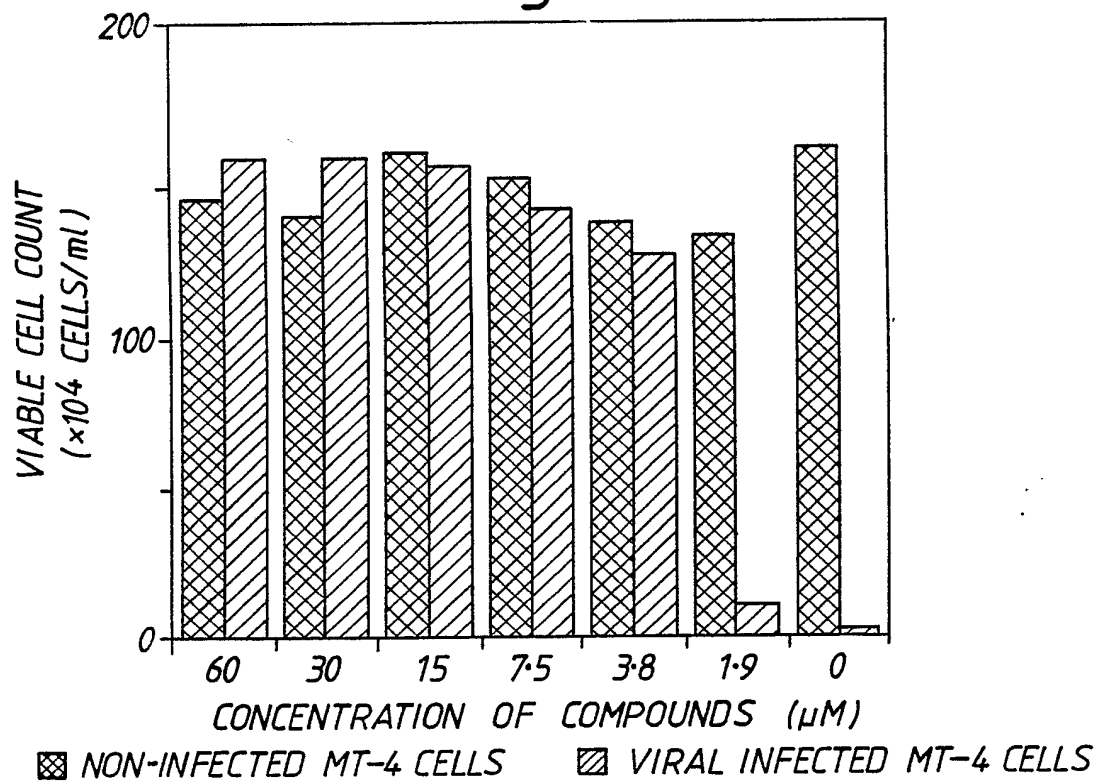
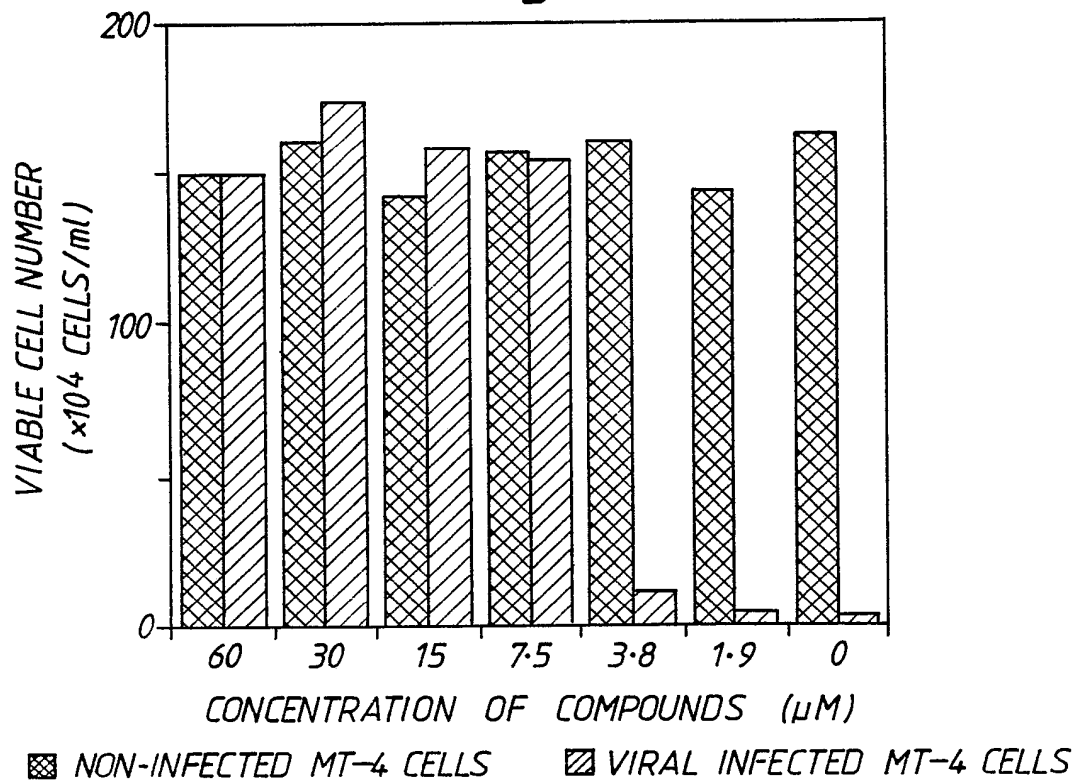
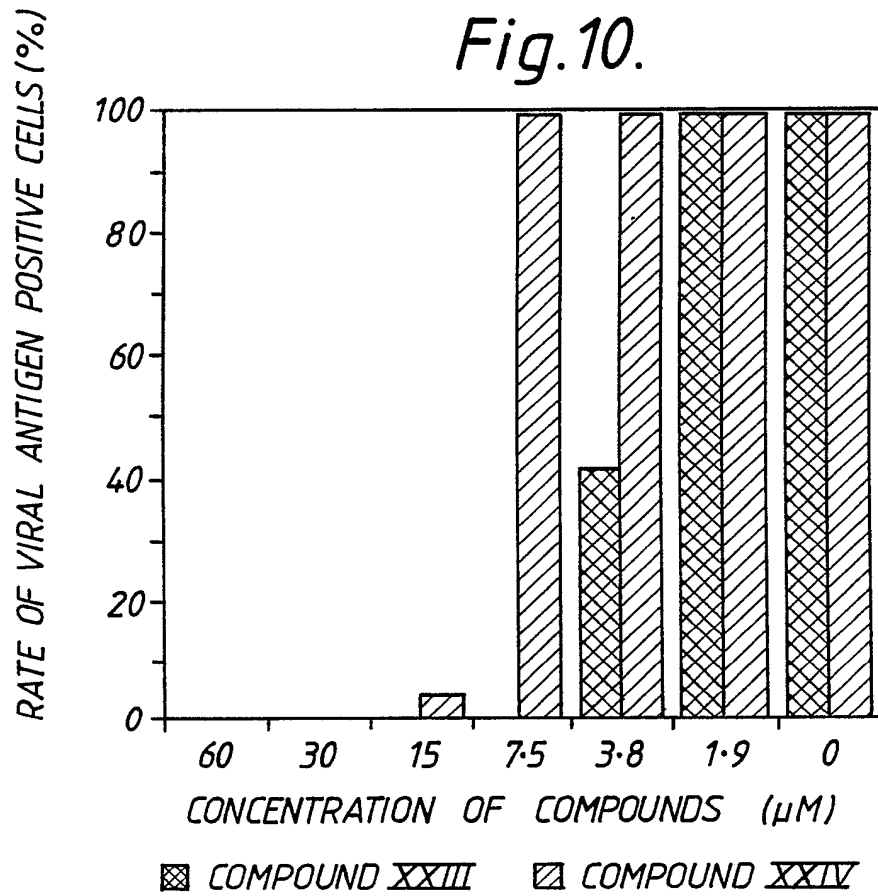


Fig. 9.





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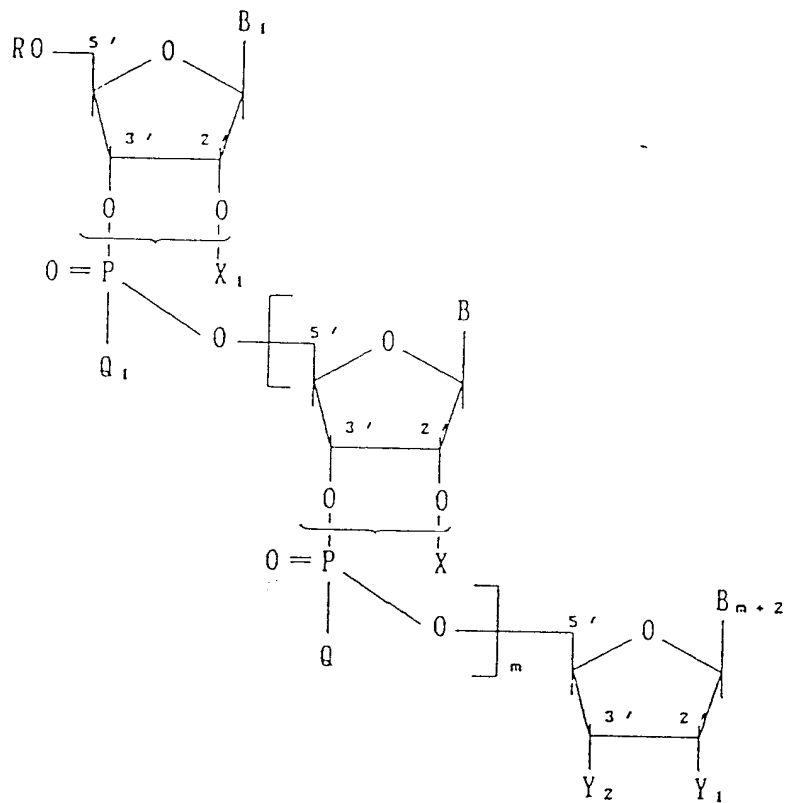
**0 339 842 A3**

(12)

**EUROPEAN PATENT APPLICATION**(21) Application number: **89303700.2**(51) Int. Cl.<sup>5</sup>: **C07H 21/00, C07H 19/067,  
C07H 19/167, A61K 31/70,  
C07J 51/00, A61K 31/58**(22) Date of filing: **13.04.89**(30) Priority: **27.04.88 JP 104943/88****06.06.88 JP 138966/88****06.07.88 JP 168142/88****12.09.88 JP 227887/88****22.09.88 JP 238481/88****02.02.89 JP 24372/89****16.03.89 JP 64058/89**(43) Date of publication of application:  
**02.11.89 Bulletin 89/44**(84) Designated Contracting States:  
**DE FR GB**(88) Date of deferred publication of the search report:  
**09.03.94 Bulletin 94/10**(71) Applicant: **AJINOMOTO CO., INC.**  
**5-8, Kyobashi 1-chome, Chuo-ku**  
**Tokyo 104(JP)**(72) Inventor: **Shibahara, Susumu Central**  
**Research Laboratories**  
**Ajinomoto Co., Inc.**  
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**Kawasaki-ku Kawasaki-shi****Kanagawa-ken(JP)****Inventor: Morisawa, Hirokazu Central**  
**Research Laboratories****Ajinomoto Co., Inc.****No. 1-1, Suzuki-cho****Kawasaki-ku Kawasaki-shi****Kanagawa-ken(JP)****Inventor: Nakajima, Hideki****No. 924-6, Oaza Kogushi****Ube-shi Yamaguchi-ken(JP)****Inventor: Yamamoto, Naoki****No. 1-7-12, Higashiobayama-cho****Ube-shi Yamaguchi-ken(JP)****Inventor: Mukai, Sachiko c/o Central****Research Laboratories****Ajinomoto Co., Inc.****No. 1-1, Suzuki-cho****Kawasaki-ku Kawasaki-shi****Kanagawa-ken(JP)**(74) Representative: **Bond, Bentley George et al**  
**Haseltine Lake & Co.**  
**28 Southampton Buildings**  
**Chancery Lane**  
**London WC2A 1AT (GB)**(54) **Novel oligoribonucleotide derivatives and application thereof to antiviral agents.**

(57) The invention provides oligoribonucleotide derivatives represented by the general formula:

**EP 0 339 842 A3**



wherein m, B, Q, X, R, Y<sub>1</sub>, Y<sub>2</sub> and X are as defined in the specification. These derivatives inhibit the proliferation of the AIDS virus.



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## EUROPEAN SEARCH REPORT

Application Number  
EP 89 30 3700

| DOCUMENTS CONSIDERED TO BE RELEVANT  |   |  |  |
|--|---|--|--|
| Category   | Citation of document with indication, where appropriate, of relevant passages   | Relevant to claim                                  | CLASSIFICATION OF THE APPLICATION (Int.Cl.4)                                 |
| X  | VIROLOGY<br>vol. 42, no. 2, October 1970, ORLANDO, FL, USA<br>pages 421 - 428<br>E. DE CLERCO ET AL 'The Anti-Viral Activity of Thiophosphate-Substituted Polyribonucleotides "In Vitro" and "In Vivo"'                                     | 1, 13, 29  | C07H21/00<br>C07H19/067<br>C07H19/167<br>A61K31/70<br>C07J51/00<br>A61K31/58 |
| Y  | * the whole document *  | 1, 13, 14, 24, 29                                  |  |
| Y  | ---   | 14-19, 21  |  |
| Y  | CHEMICAL ABSTRACTS, vol. 106, no. 19, 11 May 1987, Columbus, Ohio, US;<br>abstract no. 156829,<br>E. OTSUKA ET AL '2'-O-Methyl RNA'<br>page 726 ; column 2 ;<br>* abstract *<br>& JP-A-61 291 595 (KOKAI TOKKYO KOHO) 22 December 1986      | 1, 13, 14, 24, 29                                  |  |
| Y  | NATURE<br>vol. 313, no. 6000, January 1985, LONDON GB<br>pages 277 - 284<br>L. RATNER ET AL 'Complete Nucleotide Sequence of the AIDS Virus, HTLV-III'<br>* page 278, column 2, line 11 *<br>* page 279, column 1, line 36 *<br>---<br>-/-- | 14-19, 21  | TECHNICAL FIELDS SEARCHED (Int.Cl.4)<br><br>C07H<br>A61K                     |
| The present search report has been drawn up for all claims   |   |  |  |
| Place of search<br>THE HAGUE   |   | Date of completion of the search<br>6 January 1994 | Examiner<br>Watchorn, P  |
| <b>CATEGORY OF CITED DOCUMENTS</b><br>X : particularly relevant if taken alone<br>Y : particularly relevant if combined with another document of the same category<br>A : technological background<br>O : non-written disclosure<br>P : intermediate document<br><br>T : theory or principle underlying the invention<br>E : earlier patent document, but published on, or after the filing date<br>D : document cited in the application<br>L : document cited for other reasons<br>-----<br>& : member of the same patent family, corresponding document |   |  |  |





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### CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claims:
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

### LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions.

namely:

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- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☒ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims: Claims: 1-7, 9-13, 29-30, 33 (all in part) and 31 (whole):
- Claims: 1-5, 8, 13, 29, 30, 34 (all in part) 14-21, 24-28 (all whole)



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|--|--|--|--|--|---|
| Category   | Citation of document with indication, where appropriate, of relevant passages  | Relevant to claim                                  | CLASSIFICATION OF THE APPLICATION (Int.Cl.4) |  |   |
| A  | BIOCHEMISTRY.<br>vol. 18, no. 19 , 18 September 1979 ,<br>EASTON, PA US<br>pages 4116 - 4120<br>D. YEE ET AL 'Mechanistic Studies on<br>Deoxyribonucleic Acid Dependent<br>Ribonucleic Acid Polymerase from<br>Escherichia coli Using Phosphorothioate<br>Analogues. 1. Initiation and Pyrophosphate<br>Exchange Reactions'<br>* page 4117; figure I *<br>* page 4118, column 1, line 4 - line 14 *<br>--- | 1,13,29  | TECHNICAL FIELDS<br>SEARCHED (Int.Cl.4)      |  |   |
| A  | BIOCHEMISTRY.<br>vol. 21, no. 23 , 9 November 1982 ,<br>EASTON, PA US<br>pages 5877 - 5885<br>F. R. BRYANT ET AL 'Phosphorothioate<br>Substrates for T4 RNA Ligase'<br>* page 5880, column 2, line 24 - line 62 *<br>* page 5881, column 2, line 11 - line 34 *<br>* page 5883, column 2, line 30 - page<br>5884, column 1, line 15 *<br>---   | 1,13,29  |  |  |   |
| A  | EP-A-0 169 775 (CENTRE NATIONAL DE LA<br>RECHERCHE SCIENTIFIQUE)<br>* the whole document *<br>---  | 1,13,29  |  |  |   |
| A  | NUCLEIC ACIDS RESEARCH.<br>vol. 16, no. 4 , 25 February 1988 ,<br>ARLINGTON, VIRGINIA US<br>pages 1577 - 1591<br>A. W. NICHOLSON ET AL 'Accurate "In Vivo"<br>Cleavage by RNase III of<br>Phosphorothioate-Substituted RNA<br>Processing Signals in Bacteriophage T7<br>early mRNA'<br>* the whole document *<br>---<br>-/--   | 1,13,29  |  |  |   |
| The present search report has been drawn up for all claims   |  |  |  |  |   |
| Place of search<br>THE HAGUE   |  | Date of completion of the search<br>6 January 1994 | Examiner<br>Watchorn, P                      |  |   |
| <table><tr><td><b>CATEGORY OF CITED DOCUMENTS</b><br/>X : particularly relevant if taken alone<br/>Y : particularly relevant if combined with another<br/>document of the same category<br/>A : technological background<br/>O : non-written disclosure<br/>P : intermediate document</td><td>T : theory or principle underlying the invention<br/>E : earlier patent document, but published on, or<br/>after the filing date<br/>D : document cited in the application<br/>L : document cited for other reasons<br/>-----<br/>&amp; : member of the same patent family, corresponding<br/>document</td></tr></table> |  |  |  | <b>CATEGORY OF CITED DOCUMENTS</b><br>X : particularly relevant if taken alone<br>Y : particularly relevant if combined with another<br>document of the same category<br>A : technological background<br>O : non-written disclosure<br>P : intermediate document | T : theory or principle underlying the invention<br>E : earlier patent document, but published on, or<br>after the filing date<br>D : document cited in the application<br>L : document cited for other reasons<br>-----<br>& : member of the same patent family, corresponding<br>document |
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|--|--|--|--|-----------------------------|--|--|---|--|-----------------------|-------------------------------|---------------------------------------|------------------------------|--------------------------------------|----------------------------|-------|---------------------------|---|--|----------|
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| A  | JOURNAL OF THE AMERICAN CHEMICAL SOCIETY.<br>vol. 103, no. 3 , 11 February 1981 ,<br>GASTON, PA US<br>pages 696 - 697<br>F. R. BRYANT ET AL 'On the Mechanism of T4 RNA Ligase'<br>* the whole document *<br>---   | 1,13,29  |  |                             |  |  |   |  |                       |                               |                                       |                              |                                      |                            |       |                           |   |  |          |
| A  | NUCLEIC ACIDS RESEARCH SYMPOSIUM SERIES -<br>SEVENTH SYMPOSIUM ON THE CHEMISTRY OF<br>NUCLEIC ACID COMPONENTS HELD AT BECHYNE<br>CASTLE, CZECHOSLOVAKIA, FROM 30-08-87 -<br>05-09-87<br>no. 18 , 1987 , IRL PRESS LTD, OXFORD GB<br>pages 277 - 280<br>C. DEENEY ET AL 'Self Splicing of<br>Tetrahymena rRNA Can Proceed with<br>Phosphorothioate Substituent at the Splice<br>Sites'<br>* the whole document *<br>--- | 1,13,29  |  |                             |  |  |   |  |                       |                               |                                       |                              |                                      |                            |       |                           |   |  |          |
| A  | PROCEEDINGS OF THE NATIONAL ACADEMY OF<br>SCIENCES OF USA<br>vol. 84, no. 21 , November 1987 ,<br>WASHINGTON US<br>pages 7706 - 7710<br>M. MATSUKURA ET AL 'Phosphorothioate<br>Analogues of Oligodeoxynucleotides:<br>Inhibitors of Replication and Cytopathic<br>Effects of Human Immunodeficiency Virus'<br>* the whole document *<br>---<br>-/--   | 1,13,29  |  |                             |  |  |   |  |                       |                               |                                       |                              |                                      |                            |       |                           |   |  |          |
| The present search report has been drawn up for all claims   |  |  |  |                             |  |  |   |  |                       |                               |                                       |                              |                                      |                            |       |                           |   |  |          |
| Place of search<br>THE HAGUE   |  | Date of completion of the search<br>6 January 1994 | Examiner<br>Watchorn, P                      |                             |  |  |   |  |                       |                               |                                       |                              |                                      |                            |       |                           |   |  |          |
| <table border="0"><tr><td>CATEGORY OF CITED DOCUMENTS</td><td>T : theory or principle underlying the invention</td></tr><tr><td>X : particularly relevant if taken alone</td><td>E : earlier patent document, but published on, or</td></tr><tr><td>Y : particularly relevant if combined with another</td><td>after the filing date</td></tr><tr><td>document of the same category</td><td>D : document cited in the application</td></tr><tr><td>A : technological background</td><td>L : document cited for other reasons</td></tr><tr><td>O : non-written disclosure</td><td>.....</td></tr><tr><td>P : intermediate document</td><td>&amp; : member of the same patent family, corresponding</td></tr><tr><td></td><td>document</td></tr></table> |  |  |  | CATEGORY OF CITED DOCUMENTS | T : theory or principle underlying the invention | X : particularly relevant if taken alone | E : earlier patent document, but published on, or | Y : particularly relevant if combined with another | after the filing date | document of the same category | D : document cited in the application | A : technological background | L : document cited for other reasons | O : non-written disclosure | ..... | P : intermediate document | & : member of the same patent family, corresponding |  | document |
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|  | document   |  |  |                             |  |  |   |  |                       |                               |                                       |                              |                                      |                            |       |                           |   |  |          |



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## EUROPEAN SEARCH REPORT

Application Number  
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| DOCUMENTS CONSIDERED TO BE RELEVANT   |  |   |  |
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| Category  | Citation of document with indication, where appropriate, of relevant passages  | Relevant to claim   | CLASSIFICATION OF THE APPLICATION (Int.Cl.4) |
| P,X   | NUCLEIC ACIDS RESEARCH.<br>vol. 17, no. 1 , 11 January 1989 ,<br>ARLINGTON, VIRGINIA US<br>pages 239 - 252<br>S. SHIBAHARA ET AL 'Inhibition of Human<br>Immunodeficiency Virus (HIV-1) Replication<br>by Synthetic Oligo-RNA Derivatives'<br>* whole document, particularly page 245 *<br>----- | 1,13-19,<br>21,24,29  |  |
|   |  |   | TECHNICAL FIELDS<br>SEARCHED (Int.Cl.4)      |
|   |  |   |  |
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European Patent  
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EP 89 30 3700 -B-

### LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,

namely:

1. Claims: 1-7, 9-13, 29-30, 33 (all in part) and 31 (whole): RNA phosphothioate derivatives where the nucleotides are joined 3'-5' and there is an unsubstituted OH group in the 2' position, and pharmaceutical compositions thereof.
2. Claims: 1-7, 9-13, 29-30, 33 (all in part) and 32 (whole): RNA phosphothioate derivatives where the nucleotides are joined 2'-5' and there is an unsubstituted OH group in the 3' position, and pharmaceutical compositions thereof.
3. Claims: 1-5, 8, 13, 29, 30, 34 (all in part) 14-21, 24-28 (all whole)  
RNA phosphothioate derivatives where the nucleotides are joined 3'-5' and there is a substituted OH group in the 2' position, and pharmaceutical compositions thereof.
4. Claims: 1-5, 8, 13, 29, 30, 34 (all in part)  
RNA phosphothioate derivatives where the nucleotides are joined 2'-5' and there is a substituted OH group in the 3' position, and pharmaceutical compositions thereof.
5. Claims: 22-23 (all whole)  
Intermediates for the production of above compounds.